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— SECTION C —

BOTANICAL SCIENCES

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THE VEGETATIVE AND FROST-FREE SEASONS OF THE PRAIRIE PROVINCES AND THE NORTHWEST TERRITORIES[1]

BY B. W. CURRIE[2]

Abstract

A comparison is made of the changes in the lengths of the vegetative and frost-free seasons in the Prairie Provinces and the Northwest Territories, based on a mean daily temperature of 42° F. for growth and a minimum temperature of 32° F. for frost damage. Figures are included that show the broad details of the beginning and of the ending for both seasons. From these figures the lengths of the seasons may be deduced for various districts. A comparison is made of the average daily temperatures for the vegetative seasons at a number of representative places. The variability in the beginning and the ending of the frost-free season for a number of places is indicated by a table giving extreme, 25-percentile, 50-percentile, and 75-percentile dates. These values are shown graphically by a unique type of diagram that also shows the median length of the frost-free season.

Introduction

Considerable evidence (1; 4, pp. 36, 37; 8) exists that many plants of economic value can be successfully cultivated in the northern part of the Prairie Provinces and parts of the Northwest Territories. Except for northern Alberta, where various cereals, forage plants, fruits, and vegetables are regularly grown at places as far north as Fort Vermilion (latitude 58° 27′ N.), current and past experiences have been limited chiefly to small plots at missions and trading-posts in northern Saskatchewan and Manitoba and along the Mackenzie River. Since some increase of agricultural activities in these regions is certain to take place within the next few years in order to meet local needs for agricultural products, and since considerable areas with soils suitable for cultivation are likely to be found, criteria are required to indicate: (*a*) the hazards from the shortness of the growing season and from frost when varieties of plants are introduced from the southern part of the Prairie Provinces; and (*b*) the extent to which such varieties must be improved in hardiness and earliness of maturity for them to produce profitable crops at the higher latitudes.

In any estimation of the effect of temperature on plant growth at least two critical temperatures require consideration, namely, the temperature at which

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growth begins in the spring and ceases in autumn and the temperature at which either chilling or freezing noticeably reduces the return from the plant. While no one set of temperature values is equally applicable to all species of plants, or even to different varieties of the same species, or on all occasions to the same variety, a lack of knowledge of the exact temperatures for each case or situation and the labors of handling large accumulations of climatic data have forced the selection of some one set of values that is generally applicable to the economic plants of a particular region. For temperate climates, such as prevail in the southern part of the Prairie Provinces, a daily mean temperature of 42° F. and a minimum temperature of 32° F. (as recorded in a weather shelter) are in common use. The period between the two dates, the first in spring and the second in the autumn, when the mean daily temperatures are above the former value, is the vegetative or growing season, and the period between the average date in late spring and the average date in early autumn when the temperature is always above the latter value is the frost-free season. On an average basis, sufficient heat is available throughout the first period for the majority of economic plants that might conceivably be grown in the region to make some growth, and damage from chilling does not occur during the second period.

The Vegetative Period

A method described by Brooks (3) was used for finding the dates of the beginning and the ending of the vegetative period. This method assumes that the mean monthly temperature is the mean temperature of the middle day of the month, and that the mean temperatures for the three calendar months, covering the date required, fit on a sine curve with an annual period. An interpolation is made for the date by means of a table based on these assumptions. A check of the method was made by the direct but tedious method of averaging the daily mean temperatures for a 45 year period at Saskatoon. The check gave the same dates. On the other hand, an interpolation for the dates, based on a linear variation of the mean temperature from the middle of one month to the next, gave values differing by three and four days from the aforementioned ones.

In general, the mean monthly temperatures were determined from the monthly temperatures published by the Dominion Meteorological Service. In the case of the cultivated area of the Prairie Provinces, 50 stations were selected because of the representativeness of their temperature records for the region as a whole, and their mean monthly temperatures were reduced to values for a normal period from 1900 to 1939, inclusive. For the remainder of the Prairie Provinces and for the Northwest Territories, all available temperature records were used. Since great distances separate many of the weather stations, a reduction to a normal period was impossible. However, the number and distribution of the stations, and the lengths of most of their records are sufficiently good to determine growing periods that show spatial variations in accordance with the known factors affecting temperature.

Fig. 1 shows isolines for the dates of the beginning of the vegetative season for the Prairie Provinces and the major portion of the Northwest Territories. Most of the Arctic Archipelago is omitted because of either the inadequacy of the temperature data or the locations of the stations leading to temperatures

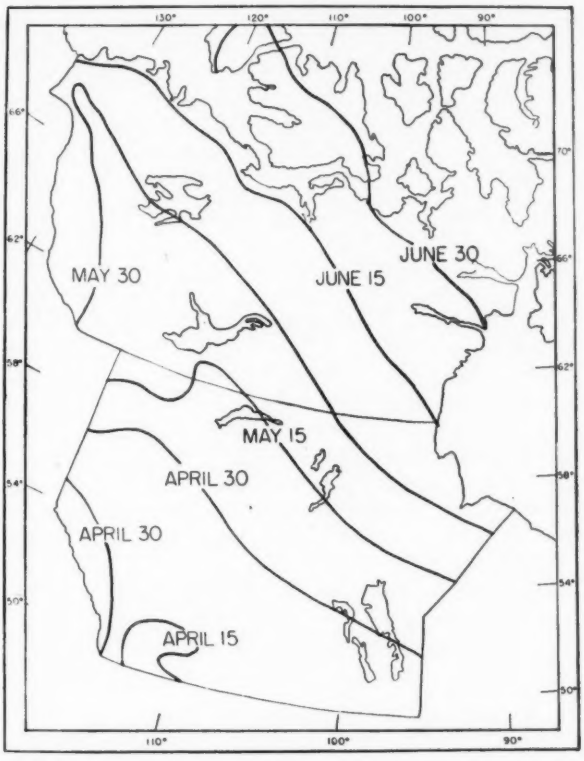

FIG. 1. *Isolines for the mean dates of the beginning of the vegetative season for the Prairie Provinces and the Northwest Territories.*

that are not typical of the surrounding regions. The isolines are drawn for periods of 15 days, a period commensurate with the representativeness of the temperature data and the scale of the map.

On the average, mean daily temperatures of 42° make their first appearance in southern Alberta, reaching Lethbridge and Medicine Hat by Apr. 15, and cross the cultivated region during the next 15 days. The advance northward is more rapid in Alberta than in Saskatchewan and Manitoba. This pattern for the advance is maintained until the Arctic coast between the delta of the Mackenzie River and Coronation Gulf is reached about two months after the start in southern Alberta. The retardation of two to four weeks in

the beginning of the vegetative season in northern Manitoba and the eastern part of the Northwest Territories, compared with the corresponding latitudes along the drainage basin of the Mackenzie, is due mostly to invasions of cold-air masses from off the ice-laden waters of the Arctic Archipelago and Hudson Bay.

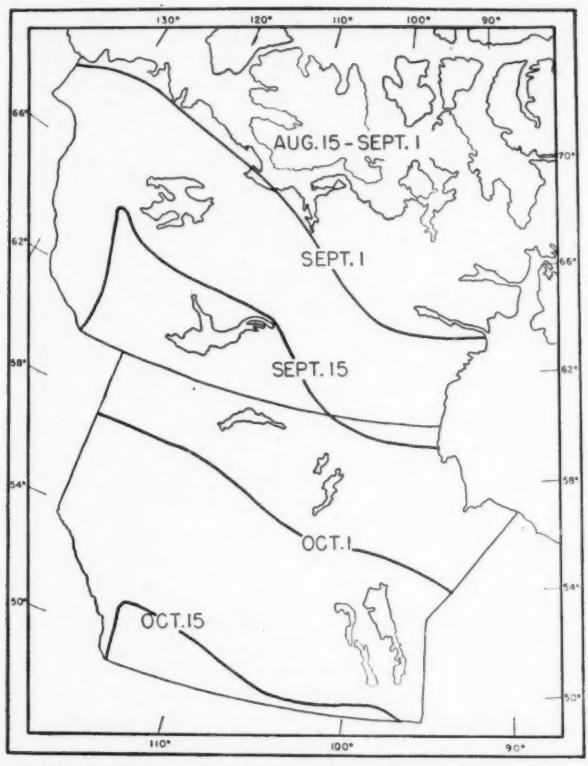

FIG. 2. *Isolines for the mean dates of the ending of the vegetative season for the Prairie Provinces and the Northwest Territories.*

Only very general statements can be made about the beginning of the vegetative period in the Arctic Archipelago. Mean daily temperatures of 42° occur along the southern coasts of Victoria and Baffin Islands during the last week of June, and not at all in the northern fringe of islands. At intermediate positions where the altitude is low and where there is some shelter from the cold winds off the ice-filled channels, they occur (if at all) sometime during the first two weeks of July. Although local conditions often become more favorable to higher temperatures after the middle of July, the steady decrease in the altitude of the sun by this time has an adverse effect; and the temperatures never rise above 42° for a significant period of time.

Fig. 2 shows isolines for the dates of the ending of the vegetative season. At places in the Archipelago with a growing period warranting consideration, the mean daily temperatures drop below 42° sometime during the last two weeks of August. A definite retreat over the mainland is in progress by Sept. 1, the period of growth having come to an end along the Arctic coast from the Mackenzie delta to Coronation Gulf and from the latter place to Chesterfield Inlet by this date. By Sept. 15, it has passed Great Bear Lake, and is approaching the eastern arm of Great Slave Lake and Churchill; by Sept. 30, it has reached the lower valley of Peace River, passed Lake Athabaska and Reindeer Lake, and is approaching Lake Winnipeg; and by Oct. 15 it is practically at the southern borders of the Prairie Provinces. The retreat parallels the lines of latitude more closely than the advance, owing to the higher water vapor content of the air masses from off the Arctic Archipelago and Hudson Bay and the consequent reduction in radiational losses of heat from the surface.

The average length of the growing season at any place may be found from Figs. 1 and 2 by using them to find the dates of the beginning and the ending, respectively, and then counting the number of days between the first and second dates. Values for a number of representative places are given in Table I. Even a casual examination of the two figures shows that the vegetative season varies from 180 to 190 days along the southern border of the Prairie Provinces to only about 90 days along a zone running northwestward from northern Manitoba to the portion of the Arctic coast located to the north of Great Bear Lake. The longest periods in the Arctic Archipelago occur in southern Victoria Island (also in southern Baffin Island) where the coastal

TABLE I

THE AVERAGE LENGTH OF THE VEGETATIVE SEASON, THE AVERAGE NUMBER OF DAY-DEGREES, AND THE AVERAGE DAILY TEMPERATURE FOR THE SEASON IN DEGREES ABOVE 42° FOR EACH OF A NUMBER OF REPRESENTATIVE PLACES IN THE PRAIRIE PROVINCES AND THE NORTHWEST TERRITORIES

Station (lat. N.; long. W.)	Vegetative season	Day-degrees	Daily temperature
	days		
Morden (49° 11′; 98° 5′)	180	2890	16.0
Lethbridge (49° 43′); 112° 51′)	189	2590	13.6
Qu'Appelle (50° 31′; 103° 56′)	175	2410	13.7
Scott (52° 22′; 108° 46′)	167	2170	13.0
Edmonton (53° 33′; 113° 30′)	174	2210	12.7
Norway House (53° 59′; 97° 50′)	149	1880	12.6
Beaverlodge (55° 10′; 119° 19′)	169	1890	11.2
Ft. Vermilion (58° 23′; 116° 3′)	141	1710	12.0
Ft. Chipewyan (58° 43′; 111° 9′)	141	1780	12.5
Churchill (58° 47′; 94° 11′)	90	740	8.2
Ft. Simpson (61° 52′; 121° 15′)	133	1560	11.7
Chesterfield (63° 20′; 90° 43′)	63	190	3.0
Coppermine (67° 45′; 115° 5′)	70	270	3.8
Aklavik (68° 14′; 134° 50′)	95	790	8.3
Cambridge Bay (69° 5′; 105° 0′)	63	200	3.2

stations have seasons of about 60 days. Since the coastal waters are cold, somewhat longer periods may exist inland in favorable localities.

It should be noted that many small areas probably exist throughout this region as a whole, where owing to either favorable or unfavorable microclimatic factors, the seasons are either longer or shorter than the ones indicated by the figures. Also, the fact that the values are primarily designed to compare temperature effects on growth should not be overlooked. Across the grasslands in the south, the growing season is often much shorter than indicated because of the lack of moisture, precipitation becoming as important a climatic factor as temperature in limiting growth. Northward, temperature rapidly becomes the predominating factor, and any shortening of the vegetative season from lack of precipitation is insignificant.

The decrease in the length of the vegetative season with increasing latitude does not indicate the corresponding change in the average daily temperature for the season, a quantity that is of some importance in estimating the possibility of maturing certain types of crops (5, pp. 292-307). One way of determining this temperature, referred to the base temperature of 42°, is to plot the mean monthly temperatures against the middle dates of the corresponding months, joining the consecutive points by straight lines, drawing the line corresponding to a constant temperature of 42°, and measuring the area enclosed by the two graphs. The area is then converted into a quantity that is nothing more than the summation of the differences between the mean daily temperature and 42° for each day in the season. This quantity is sometimes referred to as the total number of day-degrees for the season. The daily seasonal temperature in degrees above the base temperature (42°) is then obtained by dividing this summation by the number of days in the season. Although the value obtained in this way often differs by a small amount from the correct value determined by summing the differences between the mean temperature and 42° for each day and dividing this sum by the number of days in the season, it is sufficiently reliable for studies of this type and has the decided advantage that it can be obtained far more quickly. This was done for a number of representative places in the Prairie Provinces and the Northwest Territories. The average length of the vegetative season, the corresponding number of day-degrees, and the average daily temperature of the vegetative season for each place are given in Table I.

This table indicates that the number of day-degrees decreases more rapidly with latitude than the length of the vegetative season. However, at all the places in the Prairie Provinces with a continental type of climate, and even as far north as Ft. Simpson, the average daily temperatures for the growing seasons are substantially constant. At Aklavik the daily temperature is about 5° lower than for the Prairies, and is almost the same as for Churchill where the cold waters of Hudson Bay keep the seasonal temperature to a much lower value than the latitude warrants. The combined effects of high latitudes and cold waters are evident by the low seasonal values for Chesterfield and Cambridge Bay.

It is worth noting that the number of day-degrees cannot be used as an exact guide to the possibility of maturing particular crops in a given region, since the heat requirements of a plant vary with a number of other factors, such as the frequency of very high temperatures, the availability of moisture to promote growth, the hours of sunlight, etc. The long hours of sunlight at higher latitudes is often offered as a factor that annuls to some extent the effects of a shorter growing season and a lower seasonal temperature. A partial test of this idea was made from the average dates for the first seeding and the first harvesting of spring wheat at a number of the Dominion Experimental Farms (6, p. 7), and the mean monthly temperatures at these places.

TABLE II

THE AVERAGE LENGTH OF THE MATURITY SEASON FOR SPRING WHEAT AND THE CORRESPONDING NUMBER OF DAY-DEGREES AND THE AVERAGE DAILY TEMPERATURE IN DEGREES ABOVE 42° AT SOME OF THE DOMINION EXPERIMENTAL FARMS IN THE PRAIRIE PROVINCES

Station (lat. N.)	Length of season	Day-degrees	Daily temperature
Morden (49° 11′)	100	1650	16.5
Lethbridge (49° 43′)	109	1680	15.4
Swift Current (50° 20′)	108	1750	16.0
Scott (52° 22′)	107	1600	14.9
Beaverlodge (55° 10′)	119	1570	13.2
Ft. Vermilion (58° 23′)	113	1460	12.9

The appreciable increases in the lengths of the maturity seasons at higher latitudes may result from the decreases in the average seasonal temperatures. However, the known increases in effectiveness of precipitation (the ratio of actual precipitation to evaporation) at these latitudes would have the same effect. The marked decreases in the day-degrees for the maturity seasons suggest the existence of a compensating climatic factor that may well be the increased hours of sunlight.

The Frost-Free Season

Median dates were used in preference to mean dates for determining the length of the frost-free season. Trials of the two methods showed that the median values give a much better measure of the actual situation at places with a comparatively short temperature record, an extremely early or late frost altering the median date to a lesser extent than the average date. In addition, the median date is exactly the date that is far too frequently interpreted as the average date, namely, the date after or before which one-half of the years will have their last frost for the spring months, or their first frost for the autumn months. For weather stations with long records, the two dates do not differ significantly. No reduction to a normal period was

possible, since very local conditions often give large variations in the dates of frosts at stations quite close to each other.

Isolines, indicating the median dates of the last frost of spring and of the first frost of autumn in terms of days counted from the beginning of the year, are shown in Figs. 3 and 4, respectively. These are drawn for intervals of

FIG. 3. *Isolines for the median number of days from the beginning of the year to the last day in spring with frost for the Prairie Provinces and the Northwest Territories.*

10 days, a quantity commensurate with the uncertainty of the values and the scale of the maps. Isolines are not shown for the extreme northeastern portion of the mainland and most of the Arctic Archipelago, the available data (both instrumental and visual) indicating that intervals of more than 30 days without frost seldom occur there.

The northward advance of the frost-free season is similar in some respects to that of the vegetative season, the most rapid advance taking place through the drainage basin of the Mackenzie. However, only 40 days is required for the advance to reach the Arctic coast compared with 60 days for that of the

vegetative season. This results in almost a merging of the two seasons at higher latitudes. The physical cause is the long hours of sunlight at the time when the mean daily temperatures have passed 42°, and the consequent infrequency of radiational cooling reducing the minimum temperatures to 32° or lower. Local anomalies in the rate of advance are mostly due to variations

FIG. 4. *Isolines for the median number of days from the beginning of the year to the first day in autumn with frost for the Prairie Provinces and the Northwest Territories.*

of altitude. A delay of 10 to 20 days, compared with the surrounding country, occurs in the Cypress Hills of southwestern Saskatchewan; and about 10 days in the Riding and Duck Mountains and the Pasquia Hills to the west of the great lakes of Manitoba. In general, the frost-free season enters southern Alberta and Saskatchewan by May 20, crosses the grasslands by May 30, travels to the delta of Mackenzie and to the northern borders of Saskatchewan and most of Manitoba by June 19, and reaches the Arctic coasts by June 29.

The southward retreat of the frost-free season departs appreciably from the pattern followed by the vegetative season, variations of altitude, and the

moderating effects of large bodies of water (now ice free) causing large departures from even an approximate latitudinal retreat. This is most marked on the lowlands to the west of Hudson Bay and in Manitoba, where the lower altitudes and the effects of Hudson Bay and the large lakes of Manitoba cause delays of 10 to 20 days in the incidence of frost. It is also noticeable around Great Slave Lake and Lake Athabaska. Increase of altitude in the Cypress Hills brings the season to an end from 10 to 20 days earlier than in the surrounding country. In a very general way, the season ends along the Arctic coasts by Aug. 8, throughout most of the Northwest Territories by Aug. 18, and the Prairie Provinces by Sept. 10. A small part of southern Manitoba has a frost-free season that does not end until after Sept. 17. It is within this area that the larger fruits are successfully grown.

The length of the frost-free season may be found for any particular locality from Figs. 3 and 4. This is done by finding the days from the beginning of the year to the median date of the spring frost and to the median date of the autumn frost, and subtracting the former quantity from the latter. Across the south of the Prairie Provinces the season varies in length from 90 to 120 days, and in northern Alberta and to the south and west of Great Slave Lake from 70 to 90 days. From Ft. Simpson to the delta of the Mackenzie the season varies in length considerably (depending on local climatic factors) the length at Ft. Norman being 45 days, at Ft. Good Hope 57 days, and at Aklavik 65 days. Along the west coast of Hudson Bay from Churchill to Chesterfield and for some distance inland the season is approximately 70 days. At Coppermine on Coronation Gulf and at Cambridge Bay on the southeast corner of Victoria Island the season is about 60 days. Along all Arctic coasts, encumbered with ice either continuously or at frequent intervals during the summer, a year with 30 or more days free from frost rarely occurs.

Microclimatic effects result in many small areas throughout this region having frost-free seasons both notably longer and shorter than the characteristic lengths for their localities. This has been demonstrated by the observations of Albright and Stoker (2) at Beaverlodge, Alta., where they found that the frost-free season at a hilltop averaged more than three times as long as at a slough, 134 ft. lower, where the cold air drained from the surrounding slopes. At the slough frost occurred in every month of the average year, whereas on the hilltop roses bloomed not infrequently in October. At higher latitudes this drainage of cold air, resulting from radiational cooling at the surface, is not important during June or July because of the short period during the day when the outgoing radiation from the surface exceeds the incoming radiation from the sun and the atmosphere. During August the nights are considerably longer and the drainage of cold air into low-lying flats is more effective in extending the frost-free season on the higher land.

The variabilities of the dates of the last frosts of spring and the first frosts of autumn at a number of representative places were determined by finding the quartile dates for these events. These dates together with the median and extreme dates are listed in Table III. The values for each place are

TABLE III

EXTREME, 25-PERCENTILE, MEDIAN, AND 75-PERCENTILE DATES FOR THE LAST FROSTS OF SPRING AND THE FIRST FROSTS OF AUTUMN AT PLACES WHERE ECONOMIC CROPS ARE EITHER, OR MAY BE, REGULARLY GROWN. THE NUMBER BEFORE EACH STATION REFERS TO A DIAGRAM OF THE SAME NUMBER IN FIG. 5

	Station	Spring frosts					Autumn frosts				
		Earliest date	25-percentile date	Median date	75-percentile date	Latest date	Earliest date	25-percentile date	Median date	75-percentile date	Latest date
1	Morden	Ap. 23	My. 15	My. 20	My. 30	Jn. 21	Sp. 8	Sp. 15	Sp. 21	Ot. 1	Ot. 9
2	Brandon	My. 10	My. 27	Jn. 5	Jn. 12	Jl. 1	Ag. 3	Ag. 27	Sp. 6	Sp. 11	Sp. 20
3	Qu'Appelle	Ap. 19	My. 24	Jn. 4	Jn. 9	Jn. 22	Ag. 11	Ag. 27	Sp. 11	Sp. 6	Ot. 1
4	Swift Current	My. 1	My. 23	Jn. 1	Jn. 5	Jn. 22	Ag. 6	Ag. 30	Sp. 13	Sp. 23	Ot. 7
5	Medicine Hat	My. 25	My. 7	My. 14	My. 24	Jn. 6	Ag. 27	Sp. 11	Sp. 16	Sp. 24	Ot. 14
6	Berens River	My. 16	My. 26	Jn. 6	Jn. 20	Jl. 12	Ag. 14	Ag. 27	Sp. 10	Sp. 18	Ot. 4
7	Dauphin	My. 11	My. 19	My. 25	My. 30	Jn. 21	Ag. 25	Sp. 10	Sp. 16	Sp. 26	Ot. 6
8	Saskatoon	Ap. 29	My. 14	My. 22	Jn. 4	Jn. 24	Ag. 17	Sp. 7	Sp. 11	Sp. 20	Ot. 7
9	Edmonton	Ap. 29	My. 22	Jn. 1	Jn. 9	Jl. 2	Ag. 8	Ag. 28	Sp. 7	Sp. 12	Ot. 7
10	Edson	My. 30	Jn. 7	Jn. 15	Jn. 24	Jl. 9	Jl. 16	Ag. 2	Ag. 21	Sp. 3	Sp. 23
11	Beaverlodge	My. 10	My. 22	Jn. 4	Jn. 16	Jn. 27	Ag. 1	Ag. 31	Sp. 8	Sp. 13	Ot. 5
12	Dunvegan	My. 15	My. 29	Jn. 5	Jn. 15	Jn. 28	Jl. 17	Ag. 15	Ag. 27	Sp. 4	Sp. 13
13	Ft. McMurray	Jn. 1	Jn. 6	Jn. 12	Jn. 20	Jn. 29	Ag. 1	Ag. 14	Ag. 24	Sp. 2	Sp. 8
14	Waseca	My. 6	My. 20	Jn. 3	Jn. 7	Jn. 22	Ag. 2	Ag. 24	Sp. 7	Sp. 10	Sp. 17
15	Melfort	My. 10	My. 25	Jn. 5	Jn. 17	Jl. 5	Ag. 10	Ag. 26	Sp. 6	Sp. 13	Sp. 30
16	Norway House	My. 13	My. 26	Jn. 7	Jn. 14	Jl. 4	Ag. 3	Sp. 2	Sp. 10	Sp. 19	Sp. 29
17	Ft. Chipewyan	My. 22	Jn. 3	Jn. 12	Jn. 25	Jl. 1	Jl. 22	Ag. 21	Ag. 31	Sp. 6	Sp. 29
18	Ft. Smith	My. 23	Jn. 5	Jn. 18	Jn. 26	Jl. 9	Jl. 21	Ag. 8	Ag. 23	Sp. 2	Sp. 8
19	Ft. Vermilion	My. 16	Jn. 1	Jn. 11	Jn. 19	Jl. 4	Jl. 20	Ag. 8	Ag. 21	Sp. 3	Sp. 10
20	Hay River	My. 29	Jn. 5	Jn. 9	Jn. 19	Jl. 1	Jl. 31	Ag. 31	Sp. 6	Sp. 17	Sp. 28
21	Ft. Good Hope	My. 24	My. 30	Jn. 10	Jn. 20	Jl. 22	Jl. 25	Ag. 31	Ag. 6	Ag. 16	Ag. 31

Note:—Ap.—April; My.—May; Jn.—June; Jl.—July; Ag.—August; Sp.—September; Ot.—October.

shown graphically in Fig. 5 in such a way as to show also the length of the frost-free season. The upper set of diagrams is for spring frosts, and lower set for autumn frosts. The significant points in each pair of diagrams are as

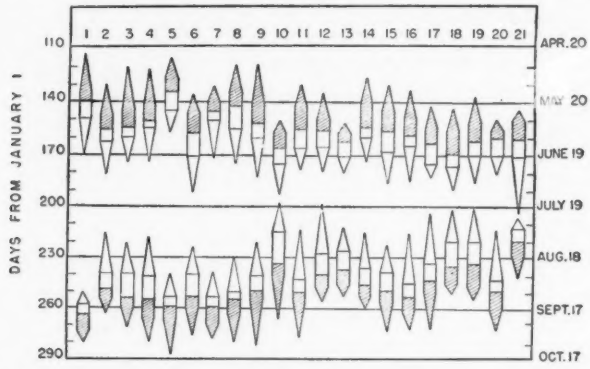

FIG. 5. *Diagrams showing extreme, 25-percentile, median, and 75-percentile dates for frosts, and the median lengths of the frost-free seasons at a number of places in the Prairie Provinces and the Mackenzie basin. The numbers at the top of the diagrams refer to place names in Table III.*

follows: (*a*) the vertices, which indicate the earliest and latest observed dates (July frosts ignored at southern stations); (*b*) the bases of the upper triangles, which indicate the 25-percentile dates or the date before which 25% of the years with observations have had their last spring frost in the case of the upper diagram and their first autumn frost in the case of the lower diagram; (*c*) the bases of the lower triangles, which indicate 75-percentile dates or the dates before which 75% of the years have had their last spring and their first autumn frost, as the case may be; and (*d*) the lines separating the shaded from the unshaded areas, which indicate the median or 50-percentile dates. The diagrams for the majority of the places are based on the observations for at least 30 years. For Qu'Appelle, Swift Current, Medicine Hat, and Edmonton, the periods are about 55 years.

From the diagrams it may be seen that the quartile differences, or the differences between the 25- and 75-percentile dates (measured in days) do not show any very characteristic variations with latitude or with locality. The differences vary from two to three weeks. In general, the two dates are not symmetrically distributed about the median date, since the intervals for the second quartile of the spring frosts and the third quartile of the autumn frosts are shorter than the intervals for the third quartile of the spring frosts and the second quartile of the autumn frosts, respectively. This grouping of the frosts is probably due to the rapid changes in temperature that occur at these times of the year,—in the first case from winter to summer values and in the second case from summer to winter values.

The majority of the frosts that take place after the median dates for the late frosts and before the median dates for the early frosts are seldom severe, shelter temperatures rarely dropping more than 3° or 4° below 32°. However, hard frosts that were decidedly unseasonal have occurred occasionally, for example, 15° at Edmonton on June 13, 1885; 21° at Swift Current on June 2, 1919; 24° at Berens River on June 26, 1927; 18° at Hay River on June 3, 1930; 19° at Ft. Smith on June 1, 1916; and 23° at Ft. McMurray on June 9, 1927. Perhaps the most untimely, widespread frost on record occurred on July 24 and 25, 1918, when temperatures as low as 20° were recorded at many places in the Peace River country, 24° in the park-belt zone of Saskatchewan, and 30° across the south of the Prairie Provinces.

The vertical spacing between the shaded sections of each pair of diagrams in Fig. 5 is proportional to the length of the frost-free season. Its steady decrease with increasing latitude is obvious. Edson (10) is an example of a place in the same latitude as Edmonton (9) but some 900 ft. higher. The effects of Great Slave Lake and Lake Athabaska in increasing the frost-free season (especially in the autumn) is apparent by comparing the sets of diagrams for Ft. Chipewyan (17) and Ft. Smith (18); and Hay River (20) and Ft. Vermilion (19), respectively.

It appears from the diagrams in Fig. 5 that exceptionally long frost-free seasons are possible, since the earlier dates of the late frosts may occur in the same year as the later dates for the early frosts. The converse situation could also occur between the late dates for the late frosts and the early dates for the early frosts. Actually, the number of observed instances are infrequent. A few typical examples for a number of places are as follows: (*a*) Lethbridge with 151 days in 1940 and 70 days in 1902; (*b*) Medicine Hat with 160 days in 1940 and 92 days in 1917; (*c*) Ft. McMurray with 100 days in 1940 and 36 days in 1934; (*d*) Ft. Vermilion with 100 days in 1941 and 47 days in 1931; (*e*) Aklavik with 107 days in 1938 and 39 days in 1934; and (*f*) Coppermine with 80 days in 1935 and 31 days in 1939. Extreme differences of this type do not occur where a large body of water acts as a control on the temperature.

Definite evidence that the frost-free season has increased in length during the past 30 years, such as has been found by Kincer (7) for the United States, does not exist. The only weather station with a long weather record that has not been affected by the growth of a large city around about it is at Qu'Appelle. Ten-year accumulated sums of the lengths of the frost-free seasons at Qu'Appelle failed to show significant trends in the length.

Acknowledgment

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THE MINERAL REQUIREMENTS FOR PYOCYANIN PRODUCTION[1]

By Margaret O. Burton,[2] Jack J. R. Campbell,[3] and Blythe A. Eagles[4]

Abstract

An investigation of the salt requirements for pyocyanin production by *Pseudomonas aeruginosa* has been carried out, and the mineral balance for maximum pyocyanin production determined. By employing a medium containing the required salt balance, it has been possible to obtain a fourfold increase in pyocyanin production over that obtained on other media usually employed for that purpose.

The ions, Mg, SO_4, K, PO_4, and Fe have been found essential to the formation of the pigment. Optimum production of pyocyanin was obtained by the use of 0.04% dipotassium hydrogen phosphate, 2.0% magnesium sulphate septahydrate, and 0.001% ferrous sulphate septahydrate in a medium containing glycerol, glycine, and leucine. In the presence of minimum amounts of Mg and SO_4, maximum pyocyanin production can be obtained by the addition of increased amounts of either Mg or SO_4.

As long as no satisfactory synthetic medium capable of providing for the production of pyocyanin by *Pseudomonas aeruginosa* was available, little work could be done on a study of the mineral requirements for the production of this pigment. The establishment of such a synthetic medium (1) has made possible a detailed study of the ionic requirements for pyocyanin formation.

Robinson in 1932 (2) determined the salt requirements for the minimum growth of *P. aeruginosa*, and, in addition, listed some of the common minerals and ions that he found unessential. He stated that both PO_4 and Mg were necessary, while Na, K, Ca, Cl, SO_4, and CO_3 were unessential. He stated further that the formation of both fluorescin and pyocyanin required only those substances necessary for growth.

In the study of the amino acid requirements for pyocyanin production (1) a salt mixture consisting of magnesium sulphate septahydrate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium chloride, and ferrous sulphate septahydrate was employed in order that the supply of minerals would not be a limiting factor in pigment formation.

In order to determine what mineral constituents of the medium are essential to pyocyanin formation and to establish the concentrations of salts required for optimum yield of the pigment the following experiments were carried out.

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Method

A synthetic medium that had been shown to be satisfactory for the production of pyocyanin by *P. aeruginosa* was employed as the basal medium. This medium designated as Medium *B* had the following composition:

	%
Glycerol	1.0
Glycine	0.6
l-Leucine	0.6
Magnesium sulphate septahydrate	0.01
Dipotassium hydrogen phosphate	0.01
Potassium dihydrogen phosphate	0.01
Potassium chloride	0.004
Ferrous sulphate septahydrate	0.0004

By omitting each salt respectively from this medium those that are required for pyocyanin formation were determined; by varying the percentage of each salt in the medium, the optimal levels of concentration of the respective salts were ascertained; and finally, by eliminating each respective ion from the medium those that are essential to pigment formation were established.

Throughout this study, water redistilled through glass was employed. All glassware was treated with nitric acid cleaning solution. Reagent quality chemicals were used.

The methods of culturing the organism (*P. aeruginosa* A.T.C. 9027) and extracting the pigment were those previously employed (1). Ten milliliter quantities of media were cultured in 125 ml. Erlenmeyer flasks and the pyocyanin after extraction with chloroform was measured in a Fischer Electrophotometer, employing suitable dilutions of the extracted pigment.

Experimental

Essential Salts

The effect of the omission of each of the component salts constituting Medium *B* on growth and pyocyanin formation is given in Table I.

TABLE I

ESSENTIAL SALTS FOR GROWTH AND PYOCYANIN PRODUCTION

Medium	Pyocyanin*	Growth
Medium *B*	40.5	++++
Medium *B* minus K_2HPO_4	24.0	++
Medium *B* minus KH_2PO_4	26.0	++
Medium *B* minus K_2HPO_4 and KH_2PO_4	0	0
Medium *B* minus $MgSO_4 . 7H_2O$	0	0
Medium *B* minus KCl	40.0	++++
Medium *B* minus $FeSO_4 . 7H_2O$	0	++++

* *Pyocyanin expressed as reading on logarithmic scale of Fischer Electrophotometer.*

It is clear from Table I that magnesium sulphate and potassium phosphate are essential to growth. The extent of growth obtained and pigment formed is shown to depend on the concentration of potassium phosphate. In the absence of ferrous sulphate maximum growth is obtained with marked production of fluorescin but no elaboration of pyocyanin. It is realized that traces of iron, sufficient for growth, were present as contaminants in the medium. However, the iron present due to contamination was calculated to be less than 0.02 p.p.m. and was shown to be inadequate for pyocyanin production. Potassium chloride would appear to be unessential for either growth or pyocyanin production in this medium.

Optimum Concentrations of Salts

Although the findings indicated that potassium chloride is not required for pyocyanin production it was thought that different concentrations of the salt might influence the quantitative production of pigment. Employing levels of potassium chloride ranging from 0.0001% to 0.1%, no effect on the production of pyocyanin was detected.

In a detailed study of the potassium phosphate requirements of the organism, dipotassium hydrogen phosphate at varying concentrations was substituted for the two phosphate salts employed in Medium *B*. The results obtained are recorded in Table II.

TABLE II

THE EFFECT OF VARYING CONCENTRATIONS OF DIPOTASSIUM HYDROGEN PHOSPHATE ON PYOCYANIN PRODUCTION

K_2HPO_4, %	Pyocyanin
0.0001	0.0
0.005	12.5
0.01	20.5
0.03	50.0
0.05	43.5
0.07	17.5
0.10	0.0

At a concentration of 0.0001% dipotassium hydrogen phosphate practically no growth was obtained and there was no pyocyanin formation. The maximum amount of pyocyanin occurred at a level of 0.03% dipotassium hydrogen phosphate. At this concentration no fluorescin was produced. At higher concentrations of phosphate the progressive decrease in pyocyanin production was accompanied by a proportional increase in fluorescin. At 0.1% dipotassium hydrogen phosphate there was good growth with marked fluorescin production but no pyocyanin formation.

The influence of magnesium sulphate septahydrate concentration on pyocyanin production is given in Table III.

TABLE III

THE EFFECT OF VARYING CONCENTRATIONS OF MAGNESIUM SULPHATE SEPTAHYDRATE ON PYOCYANIN PRODUCTION

$MgSO_4 . 7H_2O$, %	Pyocyanin
0.0001	0
0.005	29.5
0.01	45.5
0.05	59.5
0.07	60.1
0.10	61.0
0.50	60.0

Only a slight amount of growth occurred when 0.0001% magnesium sulphate septahydrate was employed. Maximum pyocyanin production was obtained between 0.05% and 0.5% magnesium sulphate septahydrate. No fluorescin was obtained under any of the experimental conditions described in Table III.

A series of concentrations of ferrous sulphate septahydrate were substituted for the 0.0004% of the salt employed in Medium *B*. The results obtained are given in Table IV.

TABLE IV

THE EFFECT OF VARYING CONCENTRATIONS OF FERROUS SULPHATE SEPTAHYDRATE ON PYOCYANIN PRODUCTION

$FeSO_4 . 7H_2O$, %	Pyocyanin
0.00001	0
0.00005	9.5
0.0001	19.5
0.0003	33.0
0.0005	45.0
0.005	47.0
0.01	22.0
0.03	0

At a concentration of 0.00001% ferrous sulphate septahydrate, abundant production of fluorescin but no evidence of pyocyanin occurred. However, as the percentage of ferrous sulphate septahydrate approached 0.0005%, fluorescin production decreased proportionally with the increase in pyocyanin. At 0.0005% and at higher concentrations, no fluorescin was observed.

Maximum pyocyanin formation occurred between 0.0005% and 0.005% ferrous sulphate septahydrate. Growth was inhibited in the presence of 0.03% ferrous sulphate septahydrate.

These experiments show that the optimum concentrations of the essential salts for pyocyanin production are 0.03% dipotassium hydrogen phosphate, 0.05% to 0.5% magnesium sulphate septahydrate and 0.0005% ferrous sulphate septahydrate. Since these optimum concentrations were determined in the presence of suboptimal concentrations of the other salts constituting the medium, it was decided to determine the effect of varying the concentrations of each of the individual salts while holding the others at their newly established optima. The salt concentrations employed and the amount of pyocyanin obtained from the respective media are recorded in Table V.

TABLE V

THE EFFECT OF SALT CONCENTRATION ON PYOCYANIN PRODUCTION

K_2HPO_4, %	$MgSO_4 . 7H_2O$, %	$FeSO_4 . 7H_2O$, %	Pyocyanin
0.03	0.1	0.0005	70.5
0.035	0.1	0.0005	69.0
0.04	0.1	0.0005	67.0
0.05	0.1	0.0005	67.0
0.03	0.3	0.0005	77.0
0.03	0.5	0.0005	84.0
0.03	1.0	0.0005	97.0
0.03	2.0	0.0005	100.0
0.03	3.0	0.0005	100.0
0.03	0.1	0.0008	71.0
0.03	0.1	0.001	71.0
0.03	0.1	0.003	77.0
0.03	0.1	0.005	62.0

The results of this experiment show that the concentrations of the salts required for the production of maximum quantities of pyocyanin lie over a wider range than previously demonstrated. The high level of magnesium sulphate septahydrate required for maximum pyocyanin production is to be especially noted. The possibility that contaminating ions present in the magnesium sulphate might be responsible for the results obtained when higher concentrations of this salt were employed was considered. However, magnesium sulphate purified by successive recrystallization from 0.05 *N* nitric acid gave identical results. Possibly owing to the relatively high concentration of iron employed in these media, no fluorescin was formed under any of the conditions outlined in Table V.

On the basis of results recorded in Table V, the optimal concentrations of the salts required for pyocyanin production were now taken as 0.04% dipotassium hydrogen phosphate, 2.0% magnesium sulphate septahydrate, and

0.001% ferrous sulphate septahydrate. Employing the salts at these levels, an experiment similar to that reported in Table V was carried out. The results confirmed the findings established in Table V as to the optimal requirements of the respective salts. A medium consisting of glycerol, glycine, and *l*-leucine in the concentrations used in Medium *B*, plus dipotassium hydrogen phosphate, magnesium sulphate, and ferrous sulphate, employed at the established optimal concentrations, gave a pyocyanin reading of 88 when a 1 : 3 dilution of the pyocyanin extract was employed, compared with a reading of 40.5 (1 : 1 dilution of pyocyanin extract) employing Medium *B*. This fourfold increase in pyocyanin production was due entirely to the alteration in the concentrations of the respective salts in the media.

Confirmation of these findings for other strains of the species was obtained when five different cultures were grown in a medium made up of salts at the concentrations established as optimum and in media containing lower and higher amounts of each of the component salts.

It was therefore concluded that, in a medium consisting of glycerol, 1%; glycine, 0.6%; and *l*-leucine, 0.6% the following salt concentrations were essential for optimum production of pyocyanin by *P. aeruginosa:*

Dipotassium hydrogen phosphate	0.04%
Magnesium sulphate septahydrate	2.0 %
Ferrous sulphate septahydrate	0.001%

Essential Ions

While dipotassium hydrogen phosphate, magnesium sulphate septahydrate, and ferrous sulphate septahydrate at certain definite concentrations have been shown to be essential for pyocyanin production the exact nature of the ions essential to pyocyanin formation has not been clearly established.

In order to establish which ions of the various salts in the basic medium required for pyocyanin production are essential, media in which one of each of the respective ions was omitted were prepared. Control media in which dipotassium hydrogen phosphate was replaced by potassium chloride and calcium monohydrogen phosphate and in which magnesium sulphate and ferrous sulphate were replaced by magnesium chloride, calcium sulphate, and ferric chloride were also prepared. The basal medium employed in this experiment was made up of glycerol, 1.0%; glycine, 0.6%; *l*-leucine, 0.6%; dipotassium hydrogen phosphate, 0.03%; magnesium sulphate septahydrate, 0.05%; and ferrous sulphate septahydrate, 0.0005%.

The pyocyanin readings of extracts obtained from these media are recorded in Table VI.

The findings show that each of the five ions employed is essential to pyocyanin formation. Good growth occurs in the absence of K, SO_4, or added Fe. In the absence of added Fe, fluorescin production without pyocyanin formation is abundant confirming the findings recorded in Table I.

As mentioned previously sufficient iron is present as contamination to support the growth of *P. aeruginosa* for Waring and Werkman (3) showed

TABLE VI

IONS ESSENTIAL TO PYOCYANIN FORMATION

Salt content of medium	Growth	Pyocyanin
1. K_2HPO_4, $MgSO_4$, and $FeSO_4$	++++	67.0
2. K_2HPO_4 replaced by KCl and $CaHPO_4$	++++	43.5
3. $MgSO_4$ and $FeSO_4$ replaced by $MgCl_2$, $CaSO_4$, and $FeCl_3$	++++	56.5
4. PO_4 omitted, K_2HPO_4 replaced by KCl	+	0
5. K omitted, K_2HPO_4 replaced by $CaHPO_4$	++++	0
6. SO_4 omitted, $MgSO_4$ and $FeSO_4$ replaced by $MgCl_2$ and $FeCl_3$	++++	0
7. Mg omitted, $MgSO_4$ replaced by $CaSO_4$	++	0
8. Fe omitted, $FeSO_4$ omitted	++++	0

that although Fe was necessary for the growth of this organism maximum growth would occur at 0.08 p.p.m. and half maximum growth at 0.02 p.p.m. It was calculated that without added iron the basal medium contained slightly less than 0.02 p.p.m. of iron and therefore would be expected to support the growth of *P. aeruginosa*.

The possibility of replacing certain of the essential ions was then considered. Unsuccessful attempts were made to substitute sodium and ammonium for potassium; manganese, zinc, or cobalt for magnesium; arsenate for phosphate; and copper for iron. In a complete medium cobalt inhibits growth and ammonium, manganese, zinc, arsenate, and copper inhibit to varying degrees the production of pyocyanin.

The marked increase in pyocyanin formation obtained with the increased concentrations of magnesium sulphate septahydrate warranted further investigation in order to ascertain whether or not increased pyocyanin production was due merely to increased growth, and also to demonstrate if possible the respective roles played by Mg and SO_4 ions in stimulating pyocyanin production.

Cultures grown in media containing a range of concentrations of magnesium sulphate septahydrate were tested for the amount of pyocyanin produced as well as for total bacterial nitrogen. The results indicated that the growth curve levelled off at 0.5% magnesium sulphate septahydrate while the pyocyanin curve continued to increase up to a 3.0% concentration of magnesium sulphate septahydrate.

By adding Mg and SO_4 ions separately in the form of magnesium chloride and sodium sulphate a more detailed picture of the relative effect of the respective ions was obtained. In the presence of 0.06% sodium sulphate, increasing amounts of Mg from 0.1% to 3.0% magnesium chloride gave tremendous increases in pyocyanin as well as increased growth. In the

presence of 0.1% magnesium chloride increases in SO_4 from 0.06% to 1.8% sodium sulphate gave similar increases in pyocyanin production but no increase in growth. These findings are in agreement with the previous observations that the SO_4 ion is required for pyocyanin production and is not essential for growth whereas Mg is essential for the optimum growth of *P. aeruginosa*. Thus Mg appeared to increase pyocyanin production by stimulating growth whereas SO_4 improves pigment production without affecting growth.

Although both Mg and SO_4 are essential to pyocyanin production, it would appear that one ion may in a measure substitute for the other in a medium designed for maximum pyocyanin production. Equal amounts of pyocyanin are produced in media containing either 0.1% magnesium chloride and 1.8% sodium sulphate; 3.0% magnesium chloride and 0.06% sodium sulphate; or 3.0% magnesium sulphate septahydrate.

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DETERMINING SEED SIZE BY A VOLUMETRIC MEASURE[1]

By Beatrice E. Murray[2]

Abstract

A method for determining seed size of flax by a volumetric measurement was devised as an alternative to weighing the seed. The volumetric or 'displacement' measurement can be expressed by means of a factor, in terms of 1000 kernel weight in grams. The 'displacement' method is more rapid than weighing the seed.

Introduction

In the breeding of flax, seed size is a factor of importance. Size of seed is usually determined on the basis of the 1000 kernel weight in grams. If many lines are to be tested, considerable time is consumed in making accurate weight determinations, using either the analytic or torsion balance, as the variations in weight are small. The volumetric or 'displacement' method was devised for the purpose of increasing efficiency in this operation.

Materials and Methods

The method of determining seed size consisted of measuring the volume of liquid displaced by a sample of 100 seeds in a glass tube 7 mm. in diameter and 16 cm. long. The tube was clamped in a vertical position to an 18 in. iron stand, using a Bunsen clamp with one flat and one V-shaped jaw. This tube, open at both ends, was used to hold a column of liquid. A stopper placed in the bottom held the liquid while the measurements were taken and enabled quick emptying and clearing of the tube. A millimeter scale was fastened with the tube in the Bunsen clamp in such a way as to allow the scale to be read through the tube.

Ethyl alcohol was used as the displacement liquid as it has a low surface tension that allowed the flax seeds to drop freely to the bottom of the tube. The alcohol quickly evaporated from the seeds and tests showed that it had no effect on germination. The alcohol could be reused when the flax seeds were strained out.

The procedure in making the seed size determinations was to place about 1 cc. of ethyl alcohol in the tube by means of a pipette. One hundred seeds were then counted out as this allowed time for the liquid to drain down from the sides of the tube. To ensure a minimum of error in measurement a plunger was used to remove any liquid adhering to the sides of the tube. The height of the column of liquid was then measured in millimeters. Following this the seeds were dropped through a small funnel into the tube and the

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Contribution from the Laboratories of the University of Saskatchewan, Saskatoon, Sask. Based on part of a thesis submitted to the Department of Field Husbandry, University of Saskatchewan, in partial fulfilment of the requirements for the degree of Master of Science, April, 1947.

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height of the liquid column was again measured. The difference between the two measurements was the 'displacement' value for the seed size of the sample. When the stopper was removed the contents drained freely from the tube.

It was found that two 'displacements' could be obtained from the use of 1 cc. of alcohol. This was done by using the final or 'displacement' reading of the first sample as the base reading of the second sample. Considerably faster recording of 'displacement' was obtained by this procedure.

TABLE I

DATA ON DISPLACEMENT VALUES, CALCULATED KERNEL WEIGHTS, AND THE 1000 KERNEL WEIGHT OF 40 FLAX LINES

Flax line number	Displacement values				Factor	Calculated kernel wt.				1000 kernel weight, gm.
	Sample numbers			Mean value		Sample numbers			Mean value	
	1	2	3			1	2	3		
1	23.6	23.7	23.9	23.7	.224	5.1	5.1	5.1	5.1	5.3
2	31.2	30.1	30.9	30.7	.212	6.7	6.4	6.6	6.6	6.5
3	24.7	26.0	25.5	25.4	.209	5.3	5.6	5.5	5.5	5.3
4	28.0	28.8	28.8	28.5	.211	6.0	6.2	6.2	6.1	6.0
5	28.7	28.5	28.1	28.4	.211	6.1	6.1	6.0	6.1	6.0
6	27.5	27.0	27.3	27.3	.220	5.9	5.8	5.8	5.8	6.0
7	25.6	25.8	25.2	25.5	.208	5.5	5.5	5.4	5.5	5.3
8	24.7	25.0	25.8	25.2	.218	5.3	5.4	5.5	5.4	5.5
9	28.8	29.2	28.3	28.8	.208	6.2	6.2	6.1	6.2	6.0
10	30.6	30.8	31.2	30.9	.217	6.5	6.6	6.7	6.6	6.7
11	30.3	29.9	29.5	29.9	.207	6.5	6.4	6.3	6.4	6.2
12	27.3	27.8	26.7	27.3	.212	5.8	5.9	5.7	5.8	5.8
13	29.0	28.6	28.3	28.6	.203	6.2	6.1	6.1	6.1	5.8
14	24.4	24.7	24.9	24.7	.231	5.2	5.3	5.3	5.3	5.7
15	24.0	22.8	22.5	23.1	.225	5.1	4.9	4.8	4.9	5.2
16	25.9	26.1	25.7	25.9	.220	5.5	5.6	5.5	5.5	5.7
17	26.0	26.4	25.4	25.9	.224	5.6	5.6	5.4	5.5	5.8
18	22.0	22.8	23.0	22.6	.208	4.7	4.9	4.9	4.8	4.7
19	24.5	24.4	23.3	24.1	.207	5.2	5.2	5.0	5.1	5.0
20	28.9	28.5	28.9	28.8	.215	6.2	6.1	6.2	6.2	6.2
21	27.0	27.0	26.6	26.9	.212	5.8	5.8	5.7	5.8	5.7
22	31.0	31.0	30.7	30.9	.210	6.6	6.6	6.6	6.6	6.5
23	29.1	29.2	29.1	29.1	.213	6.2	6.2	6.2	6.2	6.2
24	28.7	28.8	29.0	28.8	.219	6.1	6.2	6.2	6.2	6.3
25	27.6	28.2	27.3	27.7	.217	5.9	6.0	5.8	5.9	6.0
26	31.1	30.3	31.0	30.8	.211	6.7	6.5	6.6	6.6	6.5
27	31.6	32.3	32.6	32.2	.211	6.8	6.9	7.0	6.9	6.8
28	25.0	24.9	25.5	25.1	.227	5.4	5.3	5.5	5.4	5.7
29	25.8	27.0	26.3	26.4	.208	5.5	5.8	5.6	5.6	5.5
30	29.2	28.4	27.7	28.4	.211	6.2	6.1	5.9	6.1	6.0
31	29.6	31.3	30.3	30.4	.220	6.3	6.7	6.5	6.5	6.7
32	26.7	27.7	25.5	26.6	.218	5.7	5.9	5.5	5.7	5.8
33	28.2	30.2	29.9	29.4	.204	6.0	6.5	6.4	6.3	6.0
34	26.6	27.6	27.5	27.2	.213	5.7	5.9	5.9	5.8	5.8
35	28.6	28.6	28.4	28.5	.218	6.1	6.1	6.1	6.1	6.2
36	23.5	23.2	23.6	23.4	.214	5.0	5.0	5.1	5.0	5.0
37	25.2	26.3	26.1	25.9	.220	5.4	5.6	5.6	5.5	5.7
38	29.0	29.5	28.4	29.0	.214	6.2	6.3	6.1	6.2	6.2
39	27.7	29.0	28.0	28.2	.213	5.9	6.2	6.0	6.0	6.0
40	23.0	23.3	23.2	23.2	.216	4.9	5.0	5.0	5.0	5.0

As seed size is generally expressed in terms of 1000 kernel weight in grams it was desirable to determine a relationship between these two methods of obtaining size of seed. The data on 40 flax lines were used to compare the methods. 'Displacements' were taken on three 100 kernel samples of each of the 40 lines. The mean displacement value of the three samples was calculated as this value tended to offset the extremes of measurement. The 1000 kernel weight was computed from the total weights of three lots of 200 seeds each, which is the accepted procedure in the Field Husbandry Department. The results of the comparison of the two methods are given in Table I.

To get the relationship of 'displacement' and 1000 kernel weight as a measure of seed size, a factor was obtained for each line as shown in Table I. This factor was obtained by dividing the 1000 kernel weight of each line by its mean 'displacement' value. It was necessary to find a factor common to both methods of seed size determination. The average of the factors of the 40 lines gave a mean factor of 0.214. The 'calculated kernel weight' was then obtained for each line by multiplying the 'displacement' value by the factor 0.214. In order to measure the variation between the two methods, the calculated kernel weight was determined for each line for all three 'displacement' values as well as for the mean.

The correlation between 1000 kernel weight and the calculated kernel weight of the three different samples and the mean was determined. The correlation coefficient for each determination is given in Table II.

TABLE II

THE CORRELATION BETWEEN 1000 KERNEL WEIGHT IN GRAMS AND THE CALCULATED KERNEL WEIGHT OF EACH OF SAMPLES 1 2, AND 3 AND THE MEAN

Factors correlated: 1000 kernel weight and calculated kernel weight	Correlation coefficient	$t = \frac{r \times \sqrt{n-2}}{\sqrt{1-r^2}}$	5% point
Sample 1	.94	17.09	2.02
Sample 2	.93	15.67	2.02
Sample 3	.94	17.09	2.02
Mean	.95	18.88	2.02

There was a very high correlation between the two methods, the least variation being shown, as would be expected, between the mean 'calculated kernel weight' and the actual 1000 kernel weight in grams. The average difference between the calculated and the actual 1000 kernel weights was 0.135 gm. or 2.31% of the mean 1000 kernel weight of 5.85 gm. This difference is very small compared with the least significant differences usually accepted in comparing varieties and lines of flax.

The high degree of association between 1000 kernel weight and 'displacement' shows that 'displacement' could be used as an alternative method for

determining seed size of flax samples. The 'displacement' method could conveniently be used when scales for taking the weight of seed are not available. It was found to be more rapid than the analytical balance as considerable time was lost in determining weight by the latter method because variations in weight are small in flax. In using the torsion balance slight variations are less apparent owing to the small weight of each seed and the frictional drag of the scale.

In the 'displacement' method small variations can be measured, as the 'displacement' scale gives a greater number of divisions than is generally used when obtaining 1000 kernel weight with standard equipment. With the new method it may therefore be legitimate to use smaller or fewer samples for computing seed weight.

Conclusions

The 'displacement' method can be used for determining size of seed of flax samples. The seed size of a large number of samples can be determined accurately in less time by the new method than when using scales for weight determination. The displacement method is more efficient than taking kernel weight as smaller or fewer samples were found to give a good measure of size. The greater numerical differences in displacement values as compared with 1000 kernel weights gave a greater range for measurement. The new method may be useful in determining seed size of other small seeded crops.

Acknowledgments

The writer wishes to express her sincere appreciation to Dr. J. B. Harrington for his suggestions and criticisms and to Mr. Jack Whitehouse for his assistance in developing the techniques employed.

ACTINOMYCIN FROM A NEW *STREPTOMYCES*[1]

By Paul C. Trussell[2] and Edwin M. Richardson[3]

Abstract

A heretofore undescribed isolate of *Streptomyces*, capable of producing actinomycin in submerged culture, has been studied. The antibiotic substance has been identified chemically and biologically as actinomycin.

Introduction

Waksman and Tishler (4) stated that, among more than 250 strains of actinomyces isolated and tested by them, only *S. antibioticus* was able to produce typical actinomycin. Further studies by Waksman *et al.* (3) on 10,000 isolated cultures of actinomycetes revealed only two species or strains of the genus *Streptomyces* other than *S. antibioticus* capable of producing actinomycin.

During the examination of microorganisms from natural sources for antimicrobial activity a *Streptomyces* has been isolated that readily produces actinomycin in submerged culture on an inexpensive medium. This *Streptomyces* isolate, tentatively designated A9-6, has been found to exhibit cultural characters distinct from *Streptomyces antibioticus* (5) (originally named *Actinomyces antibioticus*), *Streptomyces* S-4, and *Streptomyces* 36-G, the only species or strains previously reported as producing actinomycin.

Experimental

Comparison of A9-6 and the Known Actinomycin-producing Streptomyces

A comparison was made of the growth characters of A9-6 and *S. antibioticus* on various substrates. Results are given in Table I. On all the diagnostic media, A9-6 was distinct in its manner of growth from that recorded for *S. antibioticus*. In contrast to *S. antibioticus*, A9-6 failed to liquefy gelatin, produced aerial mycelia on potato and carrot plugs, caused clearing of litmus milk, and did not form a dark pigment when grown on peptone agar.

Morphological distinctions were seen in the conidiophores, which in *S. antibioticus* are arranged in clusters with no spirals, whereas those of A9-6 are commonly spiralled and exhibit whirl formation.

The sporulating aerial mycelium of A9-6 also differentiates it from *Streptomyces* 36-G, which produces straight conidiophores arranged irregularly on the aerial mycelium (3). A further distinguishing feature is that 36-G is nonchromogenic, while A9-6 is chromogenic.

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TABLE I

COMPARISON OF CULTURAL CHARACTERS OF A9-6 AND *S. antibioticus*

Medium	*S. antibioticus**	A9-6
Gelatin	Dark brown growth on surface; patches of gray aerial mycelium. Dark pigment, diffusing into unliquefied gelatin. Liquefaction slow at first, then rapid	Slow growth around sides of tube, some subsurface, grayish white. No aerial mycelium. No pigment. No liquefaction
Potato plug	Folded, brown growth, with thin black ring on plug, fading into bluish tinge. No aerial mycelium	Fairly abundant, raised, thick, wrinkled growth in 48 hr. becomes gray-brown with beads of moisture. Gray-black spores. Orange pigment. Aerial mycelium present
Carrot plug	Cream-colored to faint brownish growth. No aerial mycelium. No pigment	Fairly abundant, slightly raised growth in 48 hr. becomes gray-brown with black spores. Drops of moisture. Orange pigment. Aerial hyphae as short spirals
Litmus milk	Thick brownish ring on surface of milk. Mouse gray aerial mycelium with greenish tinge; growth becomes brown. No reaction change; no coagulation; no clearing; whitish sediment at base of tube. Old cultures: heavy growth ring at surface, heavy precipitation; liquid brownish to black in upper portion	Gray-white to gray-green flakes adhering to sides of tube at surface. No aerial mycelium Reaction slightly alkaline; almost complete clearing in five days; grayish white sediment. In old cultures: liquid red-purple to red-brown
Czapeks' agar	Thin, whitish growth. Thin gray aerial mycelium	Scant growth, close to surface, grayish white. Short gray-brown aerial mycelium. Sporophores in whirls, some in short spirals. Spores elliptical
Peptone agar	Production of dark pigment at early stage of growth very characteristic. Growth brownish, thin, with yellow-gray to yellow-green aerial mycelium	Abundant, raised, white growth in 48 hr. Yellow to orange on under surface

* *As described by Waksman and Woodruff (5).*

A9-6 is unlike *Streptomyces* 4-S, which is allied to *S. antibioticus* in some of its morphological and cultural characters (1), in that A9-6 fails to produce a deep black zone in the aerial mycelium on synthetic media.

Preparation of Antibiotic Substance

The newly isolated *Streptomyces* A9-6 was grown by standard submerged culture methods on a number of media composed of glucose, salts, and complex organic nitrogen. The medium employed most extensively contained: glucose, 1%; soymeal, 1%; sodium chloride, 0.5%; and sodium nitrate, 0.1%.

The organism was grown in aerated fermenters of 10 liter capacity. Maximum yield of the antibiotic was obtained following four to five days' incubation,

at which time the ferment was yellow-orange in color. The thallus was then removed by filtration and the filtrate subjected to purification according to the procedure of Tishler (2). A diethyl ether soluble, petroleum ether insoluble, material was obtained as red crystals following chromatographic treatment and precipitation from hot acetone by ether and subsequently from hot ethyl acetate.

Tests were made on the crystalline material to determine its identity with actinomycin.

Identity Tests

Like actinomycin (5), the antibiotic material in broth filtrates of A9-6 is removable by activated carbon; it resists 100° C. for 30 min., and it is extractable by diethyl ether and chloroform. Like actinomycin, the biologically active material of A9-6 is stable to acid (pH 1) but unstable to 0.1 *N* sodium hydroxide.

Specific rotation values ($(\alpha)_D^{25}$) of − 260° and − 286° were obtained for two impure crystalline preparations from A9-6. These compare with a value of −320° for pure actinomycin (4). The melting point of one sample was 248° to 250° C. with decomposition, compared with decomposition at 250° C. (Waksman and Tishler (4)). On reductive acetylation the substance behaved in a similar manner to actinomycin.

Visible and ultraviolet light absorption ($E_{1\text{cm.}}^{1\%}$) for one preparation from A9-6 showed two maxima, one of 170 at 446 mμ and another of 265 at 236 mμ. Another preparation gave maxima of 160 at 446 mμ and another of 230 at 242 mμ. These compare with maxima of 200 at 450 mμ and of 216 at 241 mμ for actinomycin (4).

TABLE II

SENSITIVITY OF BACTERIA TO PURE ACTINOMYCIN AND TO A PREPARATION FROM A9-6 CULTURE FILTRATE

Concentration per 10 ml. agar	*Bacillus mycoides*		*Bacillus subtilis*		*Sarcina lutea*		*Escherichia coli*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
5 μgm.	0	0	0	0	0	0		
1 μgm.	0	0	0	0	0	0		
0.5 μgm.	0	0	0	0	0	0		
0.25 μgm.	1	1	1	1	0	0		
0.10 μgm.	3	3	3	3	0	2		
0.05 μgm.	3	3	3	3	3	3		
2 mgm.							0	0
1 mgm.							3	3

NOTE: *Growth on incubation of plates for 48 hr., at 28° or 37° C.;* 0 = *none,* 1 = *trace,* 2 = *fair,* 3 = *good.*

A = pure actinomycin (Data of Waksman and Tishler (4)).

B = preparation from A9-6.

Bacterial sensitivity was determined by the agar plate-streak method on purified material from the culture filtrate of A9-6, with Waksman's strains of *Bacillus mycoides*, *Bacillus subtilis*, *Escherichia coli*, and *Sarcina lutea*. As may be seen in Table II, the bacterial sensitivity for the antibiotic from A9-6 corresponded to that found for actinomycin (4). The three Gram-positive species were highly susceptible to both antibiotics, whereas *Escherichia coli* was relatively resistant.

TABLE III

ACUTE TOXICITY TO MICE OF A9-6 PURIFIED MATERIAL (INTRAVENOUS INJECTION OF SIX MICE AT EACH DOSAGE)

Dose, μgm./gm.	Number dead after:				
	1 day	2 days	3 days	9 days	14 days
0.15	0	0	0	0	0
0.25	0	0	0	2	2
0.50	1	3	6		
1.00	4	6			
2.00	6				

The acute toxicity in mice of an A9-6 preparation was found to parallel that of actinomycin, which is lethal at a dosage of 10 μgm. subcutaneously or intraperitoneally (4). Intravenous injection of 10 μgm. of purified material from A9-6 culture filtrate was lethal to 20 gm. mice (Table III).

Discussion

Streptomyces A9-6, which has been found to differ from actinomycin-producing organisms previously described, elaborates a substance that is very similar to the antibiotic produced by *S. antibioticus*. The crude culture filtrate of A9-6 may be processed in the same manner as that of *S. antibioticus*. Although the spectrophotometric data and rotation of the material from A9-6 are not identical with those of pure actinomycin, they approximate them and differences may be attributed to impurity of the preparations from A9-6. With respect to animal toxicity and bacterial sensitivity, actinomycin and the antibiotic substance from A9-6 are identical. It therefore appears that A9-6 is a previously undescribed strain of *Streptomyces* capable of producing actinomycin in submerged culture.

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THE ISOLATION OF A BLUE FLUORESCENT COMPOUND SCOPOLETIN, FROM GREEN MOUNTAIN POTATO TUBERS, INFECTED WITH LEAF ROLL VIRUS[1]

By W. A. Andreae[2]

Abstract

A method has been described for the isolation of a crystalline, fluorescent compound that occurs in Green Mountain potato tubers during the first year of infection with leaf roll virus. The substance was identified as 7-hydroxy-6-methoxy 1 : 2 benzopyrone (scopoletin), previously isolated by Best from tobacco plants, infected with tomato spotted wilt virus.

Introduction

Several workers (1, 5, 6), have shown that in certain varieties the cut surface of tubers, infected with leaf roll virus, exhibit a bright blue fluorescence. Sanford and Grimble (6) have associated the occurrence of this fluorescence with the presence of phloem necrosis in the infected tubers. While the leaf roll disease is transmitted from the tuber to the developing plant, the symptoms of phloem necrosis and fluorescence in the tuber are not apparent after the first year of infection. We became interested in the possible role of the fluorescent principle in virus infection and attempted its isolation and identification. A similar fluorescent phenomenon had previously been observed by Best (2) in tobacco plants. This worker found that there was a slight fluorescence in healthy tobacco plants that became very pronounced on infection with tomato spotted wilt virus. The same author published later (3) a procedure by which this fluorescent substance could be isolated in crystalline form and identified it as 7-hydroxy-6-methoxy 1 : 2 benzopyrone, scopoletin. We have modified the procedure of Best, applied it to potato tissues and obtained the same compound from potato tubers, infected with leaf roll virus.

Experimental

Green Mountain potato tubers that carried the symptoms of phloem necrosis were bisected in the dark and examined in ultraviolet light from a 100 watt BH4 mercury arc. The bright fluorescent portions of about 50 tubers were cut off. As soon as 400 gm. of the cut tissue was obtained, 250 ml. of a dilute solution of sulphuric acid (about 0.05 *N* with a pH of 3 to 4) were added and the whole was finally ground in a Waring blendor. This procedure was repeated until a total of about three liters of pulp was obtained. The mash was drained and squeezed through cheesecloth and the liquid centrifuged to free it from all suspended matter. The pulp was discarded and 400 ml. portions of the aqueous extract were shaken up four times in succession with

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Contribution from the Department of Biology, University of New Brunswick, Fredericton, N.B. and the Provincial Department of Agriculture, New Brunswick.

[2] *Research Associate in Biology.*

40 ml. of chloroform. The substance was next extracted from the combined chloroform washings. Portions of the chloroform extract (300 ml.) were shaken up five times in succession with 50 ml. of ammonium hydroxide (*M*/100 with a pH of 10). The combined aqueous extracts were acidified by adding just enough normal sulphuric acid to bring the pH value to 3 to 4. The end point could be very easily recognized by the sudden disappearance of the intense blue fluorescence. Finally the fluorescent substance was extracted from 300 ml. portions of the acidified aqueous extract by five successive washings with 50 ml. of benzene. The benzene extracts were bulked, dried over neutral calcium chloride, filtered, and chromatographed on Alumina 80-200 MM (Alumina Adsorption, Fisher Scientific Co.). The adsorption column was first washed with benzene and the substance then eluted with peroxide-free ethyl ether. As soon as the fluorescent substance appeared in the eluate, the fraction was collected separately and taken down to dryness under reduced pressure at room temperature. The crystals (about 20 mgm.) were dried in a desiccator and stored in the dark. All operations from the time the tubers were bisected to the final crystallization were carried out in the dark or in subdued light, as the substance is sensitive to light.

The substance crystallized from ether as colorless needles and fluoresced when irradiated in the ultraviolet light. The melting point was determined by two different methods. A value of 206° C. was obtained when the temperature was measured with a thermocouple on the heated stage of a microscope. Subsequent determinations were carried out with a thermometer in a heating bath, employing a capillary melting point tube. The results are reported with their stem corrections. By this method, a second sample showed a melting point at 203° C. A sample of scopoletin, isolated from the roots of *Gelsemium sempervirens*, was kindly supplied by Dr. Léo Marion, National Research Council, Canada, and showed a melting point at 204.5° to 205° C. A mixed sample from these two sources gave a distinct melting point at 203° to 204° C. Since there was no depression of the melting point these substances are probably the same. Best (3) obtained a melting point of 206° C. with the material that he isolated from tobacco plants.

An aqueous solution of the pure substance at pH 7 fluoresced moderately in daylight while an alkaline aqueous solution showed a strong fluorescence. Boiling did not destroy the fluorescence and in acid solution the fluorescence was scarcely perceptible. An aqueous solution of the substance turned green when ferric chloride was added. On addition of potassium permanganate the substance gave rise to a blue color that turned yellow on alkalinization. The crystals dissolved in concentrated nitric acid producing a yellow red solution; on addition of ammonia the color became blood red.

Best (3) has published the molecular extinction curve of the compound in ethyl alcohol that is reproduced in Fig. 1. We too have measured the molecular extinction curve in the same medium using a Beckman spectrophotometer. The readings are included in Fig. 1. Maxima occurred at 2290 Å, log E 4.20 (2285 Å, log E 4.25 Best's values); 2540 Å, log E 3.72;

2980 Å, log E 3.77; 3460 Å, log E 4.12 (3435 Å, log E 4.14, Best's values). The two molecular extinction curves agree well although our curve shows two small additional maxima that were not reported by Best.

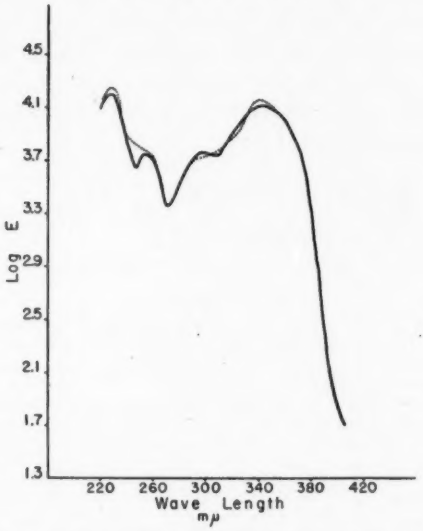

FIG. 1. *Molecular extinction curve in ethyl alcohol of the blue fluorescing compound isolated from leaf roll infected potatoes (solid line). The dotted line refers to Best's sample from tobacco infected with tomato spotted wilt and is given only where the two curves do not coincide.*

From the similarity of the physical and chemical properties of this compound with those described by Best we have concluded that the substance isolated from Green Mountain potato tubers infected with leaf roll virus is identical with the substance isolated from tobacco plants infected with tomato spotted wilt and is 7-hydroxy-6-methoxy 1 : 2 benzopyrone, scopoletin. When the procedure was applied to healthy Green Mountain tubers or to tubers past the first year of leaf roll infection, only very small amounts of the crystalline material could be obtained.

Lewis and Doty (4) partially characterized the physical and chemical properties of a blue fluorescent compound that they found to be involved in the graying or blackening process of white potatoes when the potatoes were boiled. This colorless, fluorescing pigment precursor was precipitated by ether, could be converted to a black pigment at temperatures above 35° C. or by rendering the solution alkaline, and had an absorption spectrum that showed only one characteristic maximum at 2800 Å. Evidence for the presence of a carbohydrate and a nitrogenous grouping were reported by the authors. This fluorescent substance is therefore chemically different from the one isolated by us.

Investigations on the physiological role, the origin, and the fate of scopoletin in the potato plant are in progress.

Acknowledgments

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AUTOGENOUS NECROSIS IN TOMATOES IMMUNE FROM *CLADOSPORIUM FULVUM* COOKE[1]

By Arthur N. Langford[2]

Abstract

The complication of a tomato breeding project by the occurrence, in lines carrying immunity from *Cladosporium fulvum* Cooke physiologic races 1 to 4, of a spontaneous and destructive necrosis, chiefly of the foliage, has been described.

The autogenous nature of this necrosis has been established thoroughly and the simple Mendelian nature of its inheritance determined. Immune plants, possessing the factor Cf_{P_1}, are potentially necrotic if they are homozygous with respect to the factor *ne*, found in *Lycopersicon esculentum* Mill., but remain non-necrotic if homozygous or heterozygous with respect to its dominant allele, *Ne*, found in *L. pimpinellifolium* (Jusl.) Mill. Plants susceptible to *C. fulvum* are invariably non-necrotic.

The visible expression of the gene combination Cf_{P_1}, *ne* has been shown to vary widely according to the environmental conditions, seasonal variations in the severity of necrosis being particularly striking.

The factor *ne* has been located on Chromosome I by three-point experiments. Necrosis appeared in derivatives of crosses between *L. pimpinellifolium* and all the varieties of *L. esculentum* tested.

The data indicate the presence, in *L. pimpinellifolium*, of modifying factors that reduce the severity of necrosis occurring in the presence of the gene combination Cf_{P_1}, *ne*. Evidence has also been presented that one or more factors on Chromosome VII, closely associated with the factor *H* (smooth stem), which occurs in *L. pimpinellifolium*, may prevent the development of necrosis in immune plants of genotype *ne*/*ne*, but the analysis of this phase of the problem is incomplete.

Necrosis is considered to be the visible expression of an incompatibility between Cf_{P_1} and a chromosome complex derived very largely from *L. esculentum*. Speculations concerning the possible evolution, in *L. pimpinellifolium*, of immunity from *C. fulvum* physiologic races 1 to 4 and of freedom from necrosis are outlined.

The bearing of the results of these studies upon projects for the breeding of tomatoes resistant to *C. fulvum* is discussed, particularly in the light of the discovery of races of this fungus that are capable of infecting such varieties as Vetomold, which is immune from races 1 to 4.

Introduction

The author's original investigations of the parasitism of *Cladosporium fulvum* Cooke, causal agent of tomato leaf mold, and the genetics of resistance to it began as a result of a suggestion by Dr. J. W. MacArthur of the Department of Zoology, University of Toronto, that the linkage relations of a recently discovered factor governing immunity from *C. fulvum* be determined. Dr. MacArthur provided hybrid seeds for the project, which, it was estimated, would take one summer to complete. The early discovery of additional types of resistance and of physiologic races of *C. fulvum* broadened the problem and its strictly phytopathological aspects assumed such importance that it became

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acceptable as a thesis project in the Department of Botany, University of Toronto, where the author was working under the direction of Dr. D. L. Bailey. In this manner there originated a co-operative project that has led to several lines of investigation that could not be foreseen at the time. The results of some genetical and pathological aspects of this project have been published (15, 16). It may be recalled here that the factor Cf_{p_1}, found in the red currant tomato, *Lycopersicon pimpinellifolium* (Jusl.) Mill., enables the development of immunity from *Cladosporium fulvum* physiologic races 1 to 4, and that its recessive allele, cf_{p_1}, apparently characteristic of all varieties of the ordinary cultivated tomato, *L. esculentum* Mill., permits (other resistance factors being absent) the development of plants completely susceptible to these physiologic races.

Previous to 1936, efforts to produce a tomato variety immune from *C. fulvum* occupied a minor place in the entire problem. So far as the author is aware von Sengbusch and Loschakowa-Hasenbusch (20) were the first investigators to realize the possibility of developing large-fruited esculentum-type varieties immune from *C. fulvum*. This possibility was also considered by Dr. MacArthur, who had made independent observations of the inheritance of immunity from *C. fulvum*. Dr. MacArthur suggested that immune segregants from crosses between *L. esculentum* and *L. pimpinellifolium* be crossed with large-fruited field varieties of the Earliana type, with the object of achieving large fruit size rapidly. Since it was desired to produce a variety particularly suited to our greenhouse conditions, and since the factor for immunity was known to be dominant, it seemed that it would be simpler and more satisfactory to secure the desired type by successive backcrosses of immune segregants to the English variety Potentate, which was recommended by the Horticultural Experiment Station at Vineland Station, Ont. as giving excellent performance in the greenhouse under local conditions. This procedure would obviate the necessity of eliminating by selection certain undesirable features of field tomatoes such as the frequently encountered inability to set fruit readily under greenhouse conditions. Eight successive backcrosses to Potentate would produce a line with a residue of approximately 1/256th *L. pimpinellifolium* inheritance, and it was believed that such a line would be essentially like an 'immune Potentate'. This breeding plan was adopted.

The production of the variety later named Vetomold began with a cross between Potentate and an immune F_2 segregant from a cross between *L. pimpinellifolium* and Dr. MacArthur's esculentum selection No. 705. In the first few generations only 10 to 15 plants per crop were used for this phase of the work. Successive backcrosses resulted each generation in the production of plants more esculentum-like in appearance and with greater fruit size. By the summer of 1935, however, it was evident that there might be some difficulty in establishing a line in which there did not occur a high proportion of seriously abnormal individuals. These individuals, until then found in all populations segregating with respect to immunity and susceptibility, were characterized by the appearance, after several weeks of apparently normal

development, of a spontaneous and progressive 'necrosis' of the leaves (Figs. 1 to 4). At this point the breeding program was expanded greatly and the problem of developing a variety immune from *C. fulvum* became largely one of investigating the nature of 'necrosis' and of attempting to establish lines free from this condition. The introduction of the variety Vetomold in 1939 followed the elucidation of the nature of necrosis and the establishment of immune, necrosis-free lines horticulturally very similar to the variety Potentate.

Alexander, in Ohio, and Guba, in Massachusetts, were also at work during this period incorporating the immunity of *L. pimpinellifolium* in varieties of tomato suited to their local conditions and local markets. They encountered a condition that is apparently the same as our necrosis. In 1938 Alexander (1), in reporting upon the production of the variety Globelle from a cross between *L. esculentum* var. Globe and *L. pimpinellifolium*, stated: "What appeared to be a factor for a partial lethal was observed in the F_2 generation of the original cross. Subsequent observations indicate that this factor is linked with the Cf_{p_1} resistant factor." In 1942 Guba (12), discussing the development of the variety Bay State from a cross between *L. pimpinellifolium* and *L. esculentum* var. Waltham Forcing, wrote "In the fall of 1939, a larger F_3 population was grown in several greenhouses. The performance and quality of the tomato were unusually promising but there were also some inherently bad features, notably an hereditary breakdown, manifested by yellowing, rusting, spotting, and necrosis of the foliage."

In 1942 the author (16) made a preliminary report describing briefly the nature and symptoms of necrosis and characterizing the new variety Vetomold as essentially the esculentum variety Potentate except for two dominant factors derived from *L. pimpinellifolium*: Cf_{p_1}, the immunity factor, and *Ne*, the factor preventing the development of necrosis. The factor *Ne* was located on Chromosome I.

In 1945 Clarke and Sherrard (8) discussed the appearance of symptoms of what is undoubtedly the condition described as necrosis in our studies. Necrosis appeared in their F_2 generations from crosses between Vetomold and various esculentum varieties. Clarke and Sherrard report the behavior of small populations extending to the F_4 generation, and state that their data seem to "suggest that the trouble is genetical in nature, leaf spot behaving as a recessive character".

The present belated paper constitutes a more extensive report on the nature and behavior of necrosis.

Materials and Methods

Conidia of *C. fulvum* for inoculation purposes were secured almost exclusively from infected tomato leaves from various greenhouses in the Niagara peninsula, Ontario. Previous to 1940 no attempt was made to determine which physiologic races of the fungus were present in the collections, since the factor Cf_{p_1} conferred immunity from all four physiologic races of the fungus then recognized. In the autumn of 1939, following the introduction of the

variety Vetomold, there was discovered an additional strain of *C. fulvum* capable of infecting Vetomold and other 'immune' hybrids. It thus became necessary, in connection with the necrosis problem, to obtain conidia from certain greenhouses in which the 'new' strain of the fungus had not yet appeared, as evidenced by the freedom of Vetomold from disease in the presence of a serious epiphytotic on other varieties. Nevertheless, because of the concentration of the 'new' strain built up in the Vineland greenhouses in 1940, it became increasingly difficult, during the summer months, to classify populations segregating for susceptibility to and immunity from races 1 to 4. Heavy conidial suspensions were made up in tap water just before they were to be used and both upper and lower surfaces of the leaves were heavily sprayed by means of an electrically powered DeVilbiss atomizer. After the foliage of the plants had dried the plants were transferred to a factory cotton inoculation chamber placed directly over wet soil in a greenhouse bed. Inoculations were usually carried out in the late afternoon or evening: the chamber was thoroughly wetted then and at intervals thereafter: the next afternoon the chamber was allowed to dry gradually and the plants were usually removed from it by the evening of the same day.

In preparation for the fall greenhouse crop the seeds were sown individually at the rate of 200 per seed flat (about 18 × 12 in. in size), thus allowing the seedlings to become large enough for inoculations while still in the flats, with consequent saving of space and labor. In July the usual age of the seedlings at the time of inoculation was about 16 days. Using the method outlined, 2000 or more plants could be handled at one time in the inoculation chamber. The seedlings were usually planted in pots the day after the inoculation was completed. This schedule enabled the completion of pathogenicity tests before it was time to set the plants in the soil of the field or greenhouse.

During midwinter, when the author was at the University of Toronto, or at Bishop's University, the procedure just described was found to be impracticable. The spring crop was planted before the performance of any test of immunity; crosses were made on apparently normal plants and, in the spring, after the induction of an epiphytotic, the non-necrotic plants were classified as immune or susceptible and the selection of useful crosses made upon the basis of this classification. The epiphytotic was induced in a variety of ways such as late opening and early closing of the greenhouse vents to increase the relative humidity, excessive watering, and the broadcasting of a heavy suspension of conidia by means of an electrically powered atomizer.

Unless otherwise noted all experiments were conducted at Vineland Station, Ont., latitude N. 43° 13′, longitude W. 79° 23′.

Immer's (14) tables were used for the calculation of recombination values in the linkage experiments but standard errors are used throughout the paper. The χ^2 values given are unadjusted unless otherwise stated.

Throughout the paper reference is made to various pairs of characters in the tomato by symbol only. The symbols for the recessive members of the

corresponding factor pairs, together with the characters whose development they condition, are listed at this point for reference purposes.

a_1 —green stem	*o* —elongated fruit
c —potato leaf	*p* —peach (hairy) fruit surface
cf_{p_1}—susceptibility to *C. fulvum* races 1 to 4	*r* —yellow fruit flesh
	s —compound inflorescence
d_1 —dwarf stature	*t* —tangerine orange fruit flesh
f —fasciated fruit shape	*u* —uniformly colored unripe fruits
h —hairy stem	*wt* —wilty foliage
j —jointless pedicel	*y* —clear (non-yellow) fruit epicarp
lf —leafy inflorescence	

The factor cf_{p_1} is located on Chromosome III. Table VII shows the location of the other factors. Further information concerning these factors and characters is available in articles by MacArthur (17); Butler (6), for *j* only; Langford (15), for cf_{p_1} only.

In the present studies the lineage of all plants immune from *C. fulvum* may be traced to a single sample of seed of *L. pimpinellifolium* purchased from Vaughan's Seed Store, Chicago, Ill., in 1922, under the designation 'Red Currant Tomato'.* The appearance of plants of this strain of the red currant tomato is in keeping with Muller's (19) description of *L. pimpinellifolium*.

Experimental Results

The condition here described and characterized as 'necrosis' has developed among plants that are derivatives of crosses between the ordinary cultivated tomato, *L. esculentum*, and the red currant tomato, *L. pimpinellifolium*, and retain the factor Cf_{p_1} in whose presence a plant may develop immunity** from *C. fulvum* physiologic races 1 to 4. This necrotic condition has not been observed in any other situation.

Symptomatology of Necrosis

There is considerable variation in the expression of necrosis. Although the greatest development of the condition may be seen in a crop of tomatoes grown under glass in midsummer the picture presented by an early autumn crop of tomatoes is described to show the onset of symptoms of necrosis. A summer greenhouse crop is seldom raised and plants of a spring crop comparable in degree of maturity with those of an early fall crop that is showing necrosis are themselves typically free from symptoms of necrosis. The age at which symptoms of necrosis appear in a plant varies but under our conditions plants from seed sown during the first week of July, although normal in appearance at the age of one month when grown directly in the soil under commercial conditions, typically have developed symptoms of necrosis by

* *Vaughan's have no information concerning the origin of this seed lot.*

** Note—*Hereafter immunity as used in this paper refers to immunity from Cladosporium fulvum physiologic races 1 to 4.*

the age of six weeks to such an extent that a population may be classified readily. In such plants the first clearly recognizable symptom of the abnormality is the occurrence of small, angular, yellowish to brown, necrotic spots near the tip of one or more of the lower leaves of the plant (Fig. 1). Such spots are much more apparent on the lower than on the upper surface of the leaves. The spots are not superficial and a single spot may be viewed from either the lower or the upper surface of the leaf. Accompanying the spotting is a downward and inward cupping of the leaf, essentially like that of the leaves of the potted plant shown in Figs. 2 and 3. Later symptoms consist largely of an intensification of those just described (Fig. 4). The curling of the leaf becomes more pronounced and all the leaflets typically draw together beneath the rachis. The necrotic spots become larger and more numerous, with coalescence, and plainly visible from the upper surface of the leaf as well as from the lower one. If the environmental conditions remain suitable for the development of necrosis the changes described continue, progressively, from the tip of a leaflet to its base and from distal to proximal leaflets (Fig. 3). The symptoms follow the growth of the plant upward, younger and still younger leaves successively developing the symptoms. At any time, however, the youngest growth typically remains normal in appearance (Fig. 2). Laterals that are allowed to develop are at first normal in appearance but later develop symptoms in the same sequence as does the foliage associated with the main stem. In the autumn conditions do not remain suitable for the typical development of necrosis, thus the later symptoms described are those that would be seen in the latter part of a spring crop or in a summer crop.

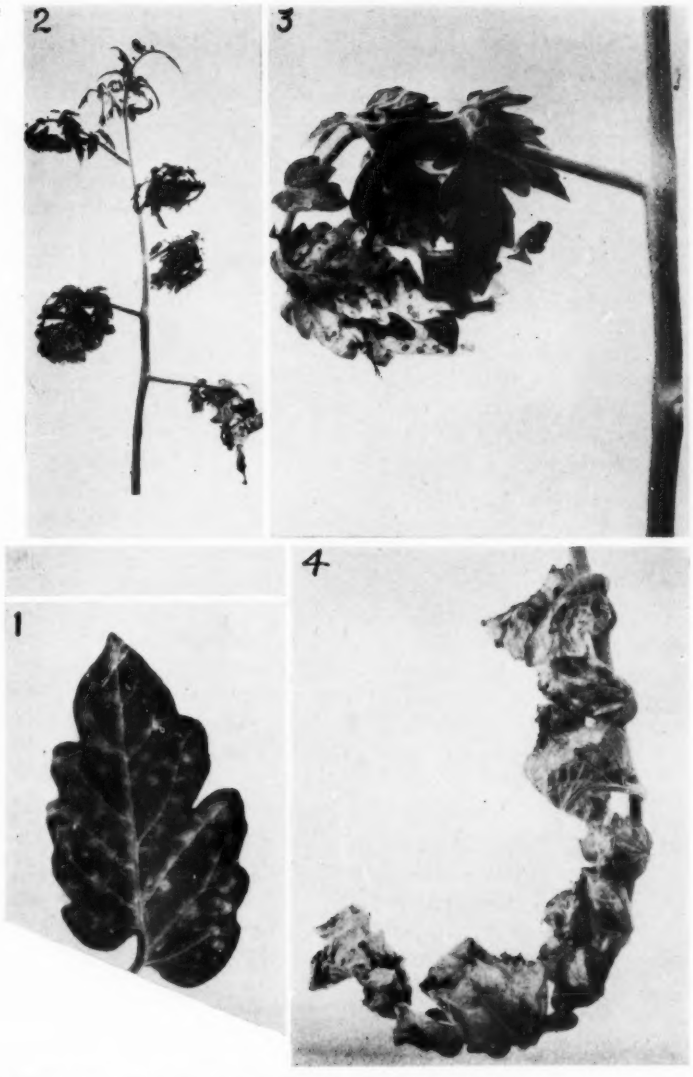
A plant in which severe necrosis has developed bears fruit of greatly reduced size. Under conditions of prolonged intense sunlight the affected plants may be completely browned except near the growing point, their leaves largely shrivelled and dead.

In the case of moderate and severe necrosis (as judged by the amount of the foliage killed), and sometimes in cases of slight necrosis, characteristic pedicel symptoms develop, especially if the truss has been well exposed to the light. The cortical tissue is necrotic: sometimes there are a few bits of green tissue but often the necrosis is so extensive that the whole truss presents a brown, killed appearance. In very severe cases the main stem may also become necrotic and in some cases necrosis is undoubtedly a factor contributing to the death of a plant in the field. In the greenhouse there has been no suggestion of an association between the presence of necrosis and the death of a plant.

The Autogenous Nature of Necrosis

Although, early in the investigation, it seemed that necrosis was of non-parasitic origin, it was considered desirable to test its supposedly autogenous nature.

Pieces of tissue including necrotic spots were cut from tomato leaves and surface sterilized with a 1 : 1000 aqueous solution of mercuric chloride and plated on potato dextrose agar and on malt agar. This procedure did not



Symptoms of necrosis on tomato plants of genotype Cf_{p_1}/Cf_{p_1}; ne/ne.

FIG. 1. *Early symptoms. Necrotic spots on upper surface of a leaflet (nine-weeks-old plant from a commercial planting, photographed Sept. 10).*

FIG. 2. *Symptoms on fifth and younger leaves of a one-month-old plant grown in a 4-in. pot (photographed Aug. 8).*

FIG. 3. *Downward and inward cupping of sixth leaf of plant photographed in Fig. 2.*

FIG. 4. *Later symptoms. Coalescence of necrotic spots and shrivelling of leaflets (nine-weeks-old plant from a commercial planting, photographed Sept. 10).*

Fig. 2, × 0.2 approximately; other Figs. × 0.6, approximately

Photographs by T. Armstrong, Dominion Entomological Laboratory, Vineland Station, Ont.

reveal any organism causally or even consistently associated with the necrotic spots. Microscopic observations of similar pieces of leaf tissue cleared in lactophenol mixture and stained with cotton blue (aniline blue) failed to show any trace of fungous mycelium in connection with the necrotic tissue.

The development of necrosis in many tomato crops indicated that it was not caused by a transmissible agent such as a virus that might be spread by pruning operations or through the activities of insects. Although symptoms of necrosis do not appear simultaneously on all potentially necrotic individuals of any one age, symptoms first appear on the plants during a reasonably short period, varying according to the season. Plants that have remained normal until necrosis has become serious on sister plants have not developed symptoms of necrosis subsequently in a single instance.

As a final check on the autogenous nature of necrosis a series of graftings and buddings was made, using combinations of necrotic and non-necrotic plants. Plants aged two and one-half months were used for this purpose on July 2. Successful combinations were made as follows, the stock being mentioned second in each instance:

Immune non-necrotic/necrotic	—two grafts and one bud
Susceptible non-necrotic/necrotic	—four grafts and one bud
Necrotic/susceptible non-necrotic	—two grafts and one bud
Necrotic/immune non-necrotic	—two grafts.

In all cases, some weeks after the union was well established, laterals from below the graft or bud level were allowed to grow. In no case was there observed any modification of the intrinsic expression of either partner of a union, with respect to the development of necrosis. Necrosis developed severely on the foliage of all potentially necrotic shoots, as on entire, sister, check plants grown at the same time in the same greenhouse bed.

The Genetics of Necrosis

The Mendelian Nature of the Inheritance of Necrosis

It has been pointed out above that necrosis develops only among certain plants possessing the immunity factor Cf_{p_1}. The data in this section show that the primary difference between immune, non-necrotic plants and immune plants having the ability to develop necrosis is due to a difference in genotype at one locus, designated as the *ne* locus. Non-necrotic plants are of the genotypes *Ne/Ne* and *Ne/ne*, homozygotes and heterozygotes being indistinguishable phenotypically; necrotic plants are of the genotype *ne/ne*. The theoretical ratios expected from various crosses, on the basis of this mode of inheritance of necrosis, are set forth in Table I. Unusual deviations from theoretical ratios and the influence of environmental factors on the development of potentially necrotic individuals are considered in later sections of this paper. The data presented in this section were obtained from populations grown under environmental conditions favoring the development of typical necrosis and in the absence of genetic factors that indicate that the

TABLE I

THE INHERITANCE OF NECROSIS IN POPULATIONS DERIVED FROM CROSSES BETWEEN *Lycopersicon pimpinellifolium* AND *L. esculentum*

Plant types crossed	Plant type numbers, plant characterizations, and genotypes of parents and progeny, with theoretical ratios of progeny			
	1. *L. pimpinellifolium* Cf_{p_1}/Cf_{p_1}; *Ne/Ne* immune, non-necrotic		2. *L. esculentum* cf_{p_1}/cf_{p_1}; *ne/ne* susceptible, non-necrotic	
1 × 2 or 2 × 1	All Cf_{p_1}/cf_{p_1}; *Ne/ne* immune, non-necrotic			
	3. F_1 of cross 1 × 2 or 2 × 1 or any plant of same genotype, derived from later backcrosses or pseudo-backcrosses Cf_{p_1}/cf_{p_1}; *Ne/ne* immune, non-necrotic		4. *L. esculentum* cf_{p_1}/cf_{p_1}; *ne/ne* susceptible, non-necrotic	
3 × 4 or 4 × 3	1 Cf_{p_1}, *Ne* : 1 immune, non-necrotic	1 Cf_{p_1}, *ne* : 1 immune, necrotic	1 cf_{p_1}, *Ne* : 2 susceptible, non-necrotic	1 cf_{p_1}, *ne*
	5. Cf_{p_1}/cf_{p_1}; *Ne/ne* any immune, non-necrotic segregant from a cross of type 3 × 4 or 4 × 3			
5 selfed or 5 × 5	9 Cf_{p_1}, *Ne* : 9 immune non-necrotic	3 Cf_{p_1}, *ne* : 3 immune, necrotic	3 cf_{p_1}, *Ne* : 4 susceptible, non-necrotic	1 cf_{p_1}, *ne*

Selfing of immune non-necrotic plants from 5 selfed or 5 × 5	Genotypes and ratios of genotypes among immune, non-necrotic parents	Theoretical ratios of progeny from the four parental genotypes		
		Immune, non-necrotic	Immune, necrotic	Susceptible, non-necrotic
	1 Cf_{p_1}/Cf_{p_1}; *Ne/Ne*	1	0	0
	2 Cf_{p_1}/Cf_{p_1}; *Ne/ne*	3	1	0
	2 Cf_{p_1}/cf_{p_1}; *Ne/Ne*	3	0	1
	4 Cf_{p_1}/cf_{p_1}; *Ne/ne*	9	3	4

simple Mendelian interpretation of the inheritance of necrosis is incomplete. The pertinent data, selected after the examination of well over 10,000 plants of segregating populations grown to maturity in the field and in the greenhouse, follow, in six parts.

1. *The Occurrence of Necrosis as Related to Immunity from Cladosporium*

Necrosis has never developed in a plant of genotype cf_{p_1}/cf_{p_1}, i.e. a plant lacking immunity from *Cladosporium*, but may develop in plants of genotype Cf_{p_1}/Cf_{p_1} or Cf_{p_1}/cf_{p_1}.

2. *Backcrosses and Pseudobackcrosses*

When immune necrotic plants derived from crosses with esculentum varieties are crossed with susceptible, esculentum-type varieties, the progeny segregates in the ratio of approximately 1 immune non-necrotic : 1 immune necrotic : 2 susceptible non-necrotic. See Table I, Cross 3 × 4, and Table II. Although Table II is based on examination of 66 populations, these were in many cases so alike in derivation that they were grouped into the 28 classes shown. Chi² tests of heterogeneity indicate the appropriateness of combining the populations in this way. The approximate values of P corresponding to the values of χ^2 calculated for the 17 groups of populations (3 $F_2$4P, 2 5GR, 6 $F_2$3P"2P, ... to 2 2P of the 1939 crop) are 0.5, 0.2, 0.7, 0.9, 0.05, 0.8, 1.0, 0.5, 0.04, 0.5, 0.3, 0.4, 0.2, 0.7, 1.0, 0.2, and 0.9, respectively. In the upper half of the table it may be seen that the pooled ratio from 14 groups of populations agrees very closely with the theoretical ratio of 1 : 1 : 2, but that the homogeneity of the populations is not very great. It will be noted, however, that the 5GR group of 1937 is the only group that does not give a satisfactory fit to a 1 : 1 : 2 ratio and also that the poor fit is due to departure from the expected ratio of 1 immune : 1 susceptible and not from the expected ratio of 1 normal : 1 necrotic. Inasmuch as the monofactorial inheritance of immunity from *Cladosporium* has been established and verified (20, 15) this deviation is not considered to be an important one. If this group is eliminated from the calculation the remaining 13 groups yield a pooled ratio of 168 : 166 : 350, with a χ^2 value of 0.385 and a P value of 0.8. The heterogeneity χ^2 thus becomes 26.172 − 9.056 + 0.385 = 17.501, with a corresponding P value of 0.1, indicating fairly satisfactory homogeneity.

In the last two columns of Table II χ^2 and P values for the immune plants only test the approach of the observed ratios to 1 : 1. The agreement with a theoretical 1 : 1 ratio is very close indeed. Two small populations deviate beyond the 5% level but since 28 populations are represented in the table the general fit is considered satisfactory. The addition of the immune plants of the upper and lower parts of Table II gives 504 normal and 513 necrotic, a χ^2 value of 0.080 and a P value of approximately 0.8. The heterogeneity χ^2 becomes 26.343 and P, with 27 degrees of freedom for the χ^2 value, approximately 0.5. It is thus clearly shown that the expectations from crosses of the type 3 × 4 in Table I have been realized satisfactorily, particularly with regard to the segregation of factors at the *ne* locus.

3. *The Selfing of Heterozygotes*

Immune non-necrotic hybrids, themselves resulting from crosses with susceptible esculentum varieties, yielded, on selfing or intercrossing, plants in the ratio of approximately 9 immune non-necrotic : 3 immune necrotic : 4

TABLE II

THE INHERITANCE OF NECROSIS IN POPULATIONS DERIVED FROM CROSSES BETWEEN HETEROZYGOUS IMMUNE, NON-NECROTIC TOMATOES AND SUSCEPTIBLE ESCULENTUM VARIETIES. SEE TABLE I, CROSS 3 × 4

Year and crop[a]	No. of populations and population designations[b]	n	Segregation ratios			χ^2 (1 : 1 : 2)	P (approx.)	χ^2 (1 : 1) immune plants only	P (approx.)
			Immune, non-necrotic	Immune, necrotic	Susc., non-necrotic				
1935, summer	1 $F_3$3P	18	6	4	8	1.111	0.6	0.400	0.5
1936, spring	1 2P	45	13	12	20	0.599	0.7	0.040	0.8
	1 3GR	18	6	4	8	1.111	0.6	0.400	0.5
	3 $F_2$4P	99	26	27	46	0.515	0.8	0.019	0.9
1937, spring	1 4P	9	3	1	5	1.000	0.6	1.000	0.3
	2 5GR	27	9	12	6	9.056	<0.01[c]	0.429	0.5
	6 $F_2$3P^{s}2P	153	36	36	81	0.529	0.8	0.000	1.0
	3 $F_2$6P	108	20	25	63	3.462	0.2	0.555	0.5
1938, spring	2 $F_2$3P^{s}2P	36	9	12	15	1.500	0.5	0.429	0.5
	2 6P	36	11	5	20	2.445	0.3	2.250	0.1
	2 7GR	36	6	10	20	1.333	0.5	1.000	0.3
	5 $F_2$8P	90	25	21	44	0.400	0.8	0.348	0.5
1939, spring	1 8P	18	4	6	8	1.111	0.6	0.400	0.5
	1 $F_2$10P	18	3	3	12	2.000	0.4	0.000	1.0
						26.172		7.270	
		711	177	178	356	0.054	0.98	0.003	0.95
			Heterogeneity χ^2 (13 degrees of freedom)			26.118	0.02[c]	7.267	0.9
1937, fall	1 5P	18	7	11				0.889	0.35
	2 6GR	32	14	18				0.500	0.5
	5 $F_2$3P^{s}3P	80	34	46				1.800	0.2
	5 $F_2$7P	102	57	45	(Susceptible plants from these populations were not grown to maturity, and the behavior of mature plants only is here reported)			1.411	0.2
1938, field	2 2P	16	12	4				4.000	0.05[c]
	1 3P	13	6	7				0.077	0.8
	1 4P	17	5	12				2.884	0.1
	1 5P	18	7	11				0.889	0.35
	3 6P	43	22	21				0.023	0.9
1938, fall	5 $F_2$3P^{s}P^{s}2P	77	40	37				0.117	0.7
	2 7P	36	16	20				0.444	0.5
	4 $F_2$9P	64	33	31				0.063	0.8
1939, field	2 2P	128	60	68				0.500	0.5
1939, fall	1 8P	18	14	4				5.556	0.02[c]
								19.153	
		662	327	335				0.097	0.8
			Heterogeneity χ^2 (13 degrees of freedom)					19.056	0.1

[a] *Spring, summer, and fall, of this column, refer to crops grown under glass.*

[b] *All populations are descended from crosses between L. pimpinellifolium and L. esculentum. The symbols P and GR refer to the esculentum varieties Potentate and Grand Rapids and the numerals preceding them indicate the number of times these varieties have been crossed into the line. Superscripts, ^{s}, ^{2s}, etc., indicate 1, 2, etc. generations of selfing. The symbol F_2 refers to a single immune F_2 segregant from a cross between L. pimpinellifolium and L. esculentum.*

[c] *Values of P less than 0.05.*

susceptible non-necrotic. See Table I, cross 5 × 5 or 5 selfed, and Table III. The ratios obtained agree reasonably well with expectation.

TABLE III

THE INHERITANCE OF NECROSIS IN POPULATIONS DERIVED FROM SELFING OR INTERCROSSING IMMUNE, NON-NECROTIC TOMATO HYBRIDS, THEMSELVES RESULTING FROM CROSSES WITH SUSCEPTIBLE VARIETIES OF *L. esculentum*. SEE TABLE I, CROSS 5 × 5 and 5 SELFED (NOTE. FOOTNOTES [a], [b], [c], OF TABLE II ALSO APPLY TO THIS TABLE)

Year and crop	No. of populations and population designations	n	Segregation ratios			χ^2 (9 : 3 : 4)	P (approx.)
			Immune, non-necrotic	Immune, necrotic	Susceptible, non-necrotic		
1936, spring	2 $F_3$3P[a]	177	102	27	48	1.532	0.5
1937, spring	1 $F_3$3P[a]P[a]	17	10	2	5	0.596	0.7
1938, field	30 $F_3$7P[a]	1689	969	304	416	0.976	0.6
						3.104	
		1883	1081	333	469	1.598	0.5
		Heterogeneity χ^2 (2 degrees of freedom)				1.506	0.5

The bottom section of Table I shows the four different genotypes that may be expected among the approximately 9/16th immune non-necrotic individuals of populations such as those recorded in Table III. Further selfing of plants of one of these genotypes, and one only, Cf_{p_1}/cf_{p_1}; *Ne/ne*, yields the phenotypic ratio 9 immune non-necrotic : 3 immune necrotic : 4 susceptible non-necrotic. Table IV includes data from all populations, including those of Table III, segregating into these three classes, regardless of their origin. The numbers of susceptible plants are not included in this table because we are primarily concerned with the *Ne–ne* factor pair and also because in many instances all of the susceptible plants of a population and some of the immune plants were discarded from 4 in. pots after the reading of their reaction to *Cladosporium*: thus, with no test of the occurrence of necrosis among the surplus immune plants, the inclusion of a figure for susceptible plants would have required the listing of fractions of plants, complicating the calculations and making no essential contribution to the analysis. Table IV shows satisfactory homogeneity of the populations grouped and of the groups of populations: the ratios of non-necrotic to necrotic plants agree well with expectation. It will be noted that nearly all of the departure from a 3 : 1 ratio in the final pooled ratio is accounted for by population 13. In view of the linkage of *ne* and d_1 part of this deficiency in necrotic individuals is believed to be referable to the shortage of about 10% from expectation among the dwarf class (d_1) in this population. See Table VII.

TABLE IV

THE INHERITANCE OF NECROSIS IN POPULATIONS DERIVED FROM THE SELFING OF HETEROZYGOUS IMMUNE, HETEROZYGOUS NON-NECROTIC TOMATO HYBRIDS, Cf_{p_1}/cf_{p_1}; Ne/ne, DERIVED FROM VARIOUS SOURCES (NOTE. FOOTNOTES [a], [b], [c] OF TABLE II ALSO APPLY TO THIS TABLE)

Population or population group number, year, and crop	Number of populations and population designations	Heterogeneity tests of grouped populations		Number of immune plants			χ^2 (3 : 1)	P (approx.)
		χ^2	P (approx.)	n	Non-necrotic	Necrotic		
1, 1936, spring	2 $F_3 3P^s$	0.158	0.7	129	102	27	1.140	0.3
2, 1937, spring	1 $F_3 3P^s P^s$			12	10	2	0.444	0.5
3, 1938, spring	1 $F_3 P^s 2P^{2s}$			23	21	2	3.260	0.08
4, 1938, spring	5 $F_2 6P^{2s}$	5.633	0.2	109	85	24	0.517	0.5
5, 1938, field	30 $F_7 7P^s$	15.724	0.98	1273	969	304	0.851	0.4
6, 1938, field	31 $F_2 6P^{2s}$	21.666	0.85	1309	954	355	3.137	0.08
7, 1939, spring	2 $7GR^{2s}$	1.028	0.3	30	24	6	0.400	0.5
8, 1937, fall	6 $F_3 P^s 2P^s$	6.847	0.25	98	73	25	0.014	0.9
9, 1937, fall	7 $F_2 6P^s$	7.745	0.25	109	80	29	0.150	0.7
10, 1938, fall	8 $F_3 3P^s P^{2s} P^s$	9.627	0.2	203	149	54	0.278	0.6
11, 1938, fall	3 $F_2 6P^{2s}$	0.753	0.7	86	63	23	0.140	0.7
12, 1938, fall	1 $7GR^s$			18	17	1	3.631	0.06
13, 1939, field	1 F_2 of ($F_2 6P^{2s}$ × *L. esculentum*)			1096	850	246	3.815	0.05
14, 1939, fall	1 7GR			18	15	3	0.667	0.4
15, 1940, field	1 F_2 of ($F_2 6P^{2s}$ × $F_2 4P^{2s}$)			106	82	24	0.314	0.6
							18.758	
				4619	3494	1125	1.022	0.3
				Heterogeneity χ^2 (14 degrees of freedom)			17.736	0.2

Figures on the numbers of susceptible plants are available not only for Populations 1, 2, and 5 of Table IV, as shown in Table III, but for Populations 3, 4, 6, and 7. The pooled ratio for these seven populations is 2165 immune non-necrotic : 720 immune necrotic : 954 susceptible non-necrotic. The χ^2 (9 : 3 : 4) value is 0.054 and the probability value, P, is 0.98. These figures fit the theoretical ratio very closely.

4. *Second Generation Selfing*

Selfing of the immune non-necrotic individuals of populations arising from the selfing of plants presumed to be of the genotype Cf_{p_1}/cf_{p_1}; Ne/ne yielded

TABLE V

THE INHERITANCE OF NECROSIS IN 74 POPULATIONS DERIVED FROM SELFING THE IMMUNE, NON-NECROTIC INDIVIDUALS IN POPULATIONS FROM THE SELFING OF TOMATO PLANTS OF THE GENOTYPE Cf_{p_1}/cf_{p_1}; *Ne/ne*. SEE ALSO THE BOTTOM SECTION OF TABLE I

Class of population	Segregation ratios expected			No. of populations with approximately the ratios shown		χ^2 (1 : 2 : 2 : 4)
	Immune, non-necrotic	Immune, necrotic	Susceptible, non-necrotic	Expected	Observed	
A	1	0	0	8.20	9	0.078
B	3	1	0	16.45	13	0.724
C	3	0	1	16.45	21	1.259
D	9	3	4	32.90	31	0.110
				χ^2 (3 degrees of freedom) = 2.171		
				P = 0.5		

results in accordance with the expectations shown in the bottom section of Table I. These results are set forth in Table V. The pooled ratios among the 13, 21, and 31 populations of Classes *B*, *C*, and *D*, respectively, of Table V, agree well with the expectations shown at the left of the table, as follows:

Class *B*—563 immune non-necrotic : 190 immune necrotic (P = 0.8).

Class *C*—912 immune non-necrotic : 1 immune necrotic (obviously a misplaced plant, and not included in the calculation) : 300 susceptible (P = 0.7).

Class *D*—954 immune non-necrotic : 355 immune necrotic : 445 susceptible non-necrotic (P = 0.2).

5. *Breeding Behavior of Necrotic Plants*

It is apparent that necrotic plants may be of the genotypes Cf_{p_1}/Cf_{p_1}; *ne/ne* and Cf_{p_1}/cf_{p_1}; *ne/ne*. Inasmuch as there was no interest in breeding a quantity of necrotic lines and since their behavior was so very obvious very little data were accumulated concerning them. However, as would be expected, the selfing of a necrotic heterozygous immune plant or the crossing of it to an esculentum variety yielded, in both instances, immune plants 100% of which were necrotic and susceptible plants that were non-necrotic. A few races of homozygous immune necrotic types have been established and inbreeding of such races has produced immune necrotic plants only.

6. *Homozygous Immune Non-necrotic Lines*

Any plant of a population whose members were all immune from *C. fulvum* and non-necrotic (populations of Class A, Table V) remained true to this type on inbreeding. Various lines of this type have been established, one of which is the variety Vetomold, introduced jointly in 1939 by the Ontario Horticultural Experiment Station and the University of Toronto. Vetomold is of the genotype Cf_{p_1}/Cf_{p_1}; *Ne/Ne*.

Environmentally Induced Variations in the Expression of Necrosis

The data presented in the preceding section, showing very clearly the simple, Mendelian nature of the inheritance of necrosis, were obtained from populations grown under conditions favoring the development of typical necrosis. The importance of the role of the environment in the development of necrosis has been shown repeatedly during the course of the investigation: a few illustrations follow.

Seasonal variations in the severity of necrosis have been very conspicuous. In general, the development of necrosis is most severe when greenhouse temperatures and the intensity of incident light are high. Plants of a line that develops severe necrosis during midsummer may appear normal during midwinter unless they are grown in a section of the greenhouse in which incident light intensity is unusually high. At Vineland Station the greenhouses are laid out on a north–south axis with large vertical exposures of glass at their southern ends. During midwinter genetically necrotic plants in the most southerly row have developed moderate to severe necrosis while similar plants in the center of the same house could not, with certainty, be identified as necrotic. In a spring crop of tomatoes, from seed sown between late December and mid-January, symptoms of necrosis are delayed greatly but appear with the coming of bright spring weather and higher temperatures, and at maturity the necrotic plants are seriously impaired functionally, producing a light yield and presenting, ultimately, a generally brown appearance. In a fall crop, from seed sown about July 11, the first symptoms of necrosis may develop characteristically by the age of about six weeks but usually do not become serious and after September new growth remains practically normal in appearance. Under these conditions the yield from genetically necrotic plants is not significantly lowered.

The time of sowing seed for the fall crop has been found to be critical with respect to the development of symptoms of necrosis. For many years July 11 has been the normal date for sowing seed for the fall crop at Vineland Station. Advancing this date a week or more has made the clear phenotypic segregation of necrotic and normal individuals a certainty whereas postponement of the sowing date for a week or more has resulted in the development of nearly normal plants that have proved difficult to classify.

Summer greenhouse crops have been grown year after year and in these the development of necrosis has been striking and severe, even in houses that had been coated with whitewash. The seriousness of the necrosis problem was, indeed, borne in to us through the observation of certain small greenhouse populations grown in the summer during the earlier stages of the development of the tomato leaf mold problem.

Typical necrosis developed in field plants of appropriate families. A population of 573 plants, for instance, from seed sown on May 31, 1940, was inoculated at age 17 days, and set out in the field at age 42 days, following a rainfall

of 1.16 in., unusual in this district. On Aug. 21, at age 82 days, necrosis, expected to appear in approximately half of the plants, was plainly visible and developing rapidly. Two days later a classification was made with ease, by simple and rapid examination of the gross appearance of the plants. A final, careful check two weeks later showed that, of the 266 necrotic individuals of this population, eight only had been missed in the Aug. 23 classification. The hours of sunshine for the months of June, July, and August were 230.9, 319.2, and 273.6, respectively, with total sunshine for the three months 6.3 hr. above the 31 year average: the monthly mean temperatures (averages of the daily means) for the same months were 63.1°, 70.5°, and 68.8° F., respectively, these averages being 1.8°, 0.8°, and 1.1°, respectively, below the 31 year average.

A second population from seed sown on June 24, 1940, and expected to yield a ratio of 1 non-necrotic : 1 necrotic, showed 360 non-necrotic and 221 necrotic plants, χ^2 being 33.26 and P far less than 0.01. This was a hybrid population segregating for various genetic factors, some of them linked with *ne*, and it is suspected that some of the deficiency in necrotic individuals is due to genetic causes. The data available are not sufficient to enable more accurate reference of this deficiency to environment and to heredity. However, data from this and other populations indicate that in the field as in the fall greenhouse crop, postponement of the sowing date results in the development of more nearly normal plants. Variations in the vigor of individual plants may account in part for the deficiency of necrotic individuals in a late field crop. It has been observed again and again that the greater the vigor of a plant the less the severity of necrosis. In connection with fall greenhouse crops surplus plants are often allowed to remain in 4 in. pots for some time after the crop has been planted. In these circumstances it has been noted that the less vigorous potted plants of a 100% necrotic line develop necrosis much earlier than do the sister plants set in the open soil, and the symptoms become more severe. The one-month-old plant photographed for Figs. 2 and 3, for instance, was grown in a 4 in. pot. It showed considerable development of necrosis, whereas the foliage of sister plants set in the soil of the greenhouse was still normal in appearance.

The effect of shading potentially necrotic individuals has been tested twice. Table VI summarizes the results of an experiment with a spring crop of tomatoes. The plants used were from an inbred line yielding 100% necrotic plants under suitable conditions. Seedlings were planted in 4 in. pots at age two weeks and, in a very vigorous, healthy condition, transplanted to 8 in. pots at age nine weeks, at which time 15 plants were moved from the open bench of greenhouse No. 1 to that of greenhouse No. 2. The shade of No. 2 was provided by a heavy, lattice-type blind. Two weeks later characteristic symptoms of necrosis were developing, particularly in the unshaded greenhouse No. 1. In greenhouse No. 2 two plants were symptomless and three practically free from necrosis; in No. 1 one plant was characterized as 'severely necrotic', its lowermost three leaves shrivelled and necrosis apparent to the

TABLE VI

THE EFFECT OF SHADING ON THE DEVELOPMENT OF NECROSIS IN POTENTIALLY NECROTIC TOMATO PLANTS GROWN FROM SEED SOWN AT TORONTO, ONT., ON JAN. 22, 1937

Greenhouse number and conditions under which the plants were grown after removal from locations *A* and *B*[a] at age 11 weeks	Number of plants selected from location		Sypmtoms of necrosis after $7\frac{1}{2}$ weeks in new locations (Column 1)
	A	*B*	
No. 1, unshaded; open bench	4	1	All severe
No. 2, heavily shaded; open bench	4	3	Two slight, three moderate, two severe
No. 2, heavily shaded; plants grown in black broadcloth cage	4	3	Practically non-necrotic but plants weak and spindly
No. 3, unshaded; open bench	2	8	All severe

[a] *Location A. Greenhouse No. 1, unshaded; open bench.*
Location B. Greenhouse No. 2, heavily shaded on east side; open bench.

10th leaf. The other plants showed a considerable range of symptoms, the degrees of necrosis designated as from exceedingly slight to moderate. A representative plant showing 'slight necrosis' had 115 small necrotic spots on the third true leaf, fewer on leaf two, but none on leaf four. Six plants from greenhouse No. 1 were at approximately this stage of development, others showed more necrosis or less necrosis than these plants. Such variation in the speed of development of symptoms of necrosis has been observed repeatedly, even in lots which, like the family here used, had been treated as uniformly as possible. The disposition of these 11-week old plants is shown in columns 1 to 3 of Table VI. The black broadcloth cage to which some plants were transferred was about 6 ft. × $3\frac{1}{2}$ ft. × 4 ft. high, open at the top, and placed on a gravel-covered bench in the center of the shaded greenhouse No. 2, in which incident light was reduced still further by running down a second heavy blind on the west side. Table VI shows clearly that reduction of light prevented the development of typical symptoms of necrosis. It may be added that the single plant earlier characterized as severely necrotic was placed in the cage and developed scarcely any symptoms of necrosis on its new foliage, whereas the two plants that were symptomless at the age of 11 weeks were placed in greenhouses No. 1 and No. 3 and both developed severe necrosis. Coincident with this experiment other segregating lines were grown in greenhouses Nos. 1 and 3. A typical, clear-cut segregation of non-necrotic and necrotic plants was evident in these populations.

The effect of shading on the development of necrosis in a fall crop was tested in 1939 in a commercial-type greenhouse at Vineland Station. Seed of a potentially necrotic line was sown on July 5 and the seedlings were planted in 4 in. pots on July 22, and protected from direct sunlight by a U-shaped formation of three seed flats placed on end. On Aug. 11 they were planted

in the most southerly row of the greenhouse and here protected from direct sunlight by a heavy shield of black broadcloth to the east, south, and west of them. Sister plants were planted in the same row, but exposed to full sunlight. On Aug. 26, by which time symptoms of necrosis were fairly general throughout the greenhouse, the four shaded plants, although noticeably spindly, showed no necrosis, while all of the other potentially necrotic plants in the same row showed unmistakable necrosis. The author soon returned to his winter work at Bishop's University and thus did not have the opportunity of watching these plants throughout the entire season. However, severe necrosis developed on the plants exposed to full sunlight, all except the uppermost leaves becoming brown and shrivelled whereas no such effects were observed on the shaded plants. That the symptoms on these exposed plants were not due simply to the high incidence of light on the plants of this most southerly row of the greenhouse, parallel to a large vertical plane of glass, was shown by the development of normal foliage on the individuals of non-necrotic lines interspersed in the row as checks.

These data show clearly that the expression of the factor combination Cf_{j1}, *ne* is very plastic: no further experiments have been undertaken to evaluate the roles of temperature and light in the development of symptoms of necrosis.

The Behavior of Populations from Various Crosses Between *L. esculentum* and *L. pimpinellifolium*

It seemed possible that necrosis might develop among the descendants of crosses between *L. pimpinellifolium* and some but not other varieties of *L. esculentum*. Accordingly crosses were made between *L. pimpinellifolium* and the widest variety of esculentum types conveniently available. The varieties used were Acme, Banalbufar, Beefheart, Bonny Best, Break O'Day, Crackerjack, Earliana, Early Detroit Purple, First of All, Grand Rapids, Imperial Globe, Potentate, Rouge Naine Hative, Sandblast, Tangerine, Tuckswood Favourite, and Wonder of Italy, most of these varieties being obtained from Dr. J. W. MacArthur's collection. In all instances the F_1 generation plants were immune from *C. fulvum* and non-necrotic. In all cases the F_2 populations showed a segregation of immune individuals into non-necrotic and necrotic individuals. In many instances the ratios gave a poor fit to a theoretical ratio of 3 normal : 1 necrotic among the immune plants (See Table IX and p. 55) but the data convinced us that necrosis would almost certainly be encountered among the descendants of any cross between *L. esculentum* and *L. pimpinellifolium*. In this connection the behavior of Dr. MacArthur's selection No. 32-13, a linkage-tester of genotype a_2, d_1, d_2, *f*, *lf*, *j*, *br*, *wt*, cf_{p1}, is of interest. A very large F_2 population from a cross of 32-13 selection with a selection of genotype Cf_{p1}/Cf_{p1}; *Ne*/*Ne* was grown to test the linkage relations of the factor *ne*. Not a single necrotic plant appeared in this population, thus showing that selection 32-13 was of the genotype cf_{p1}/cf_{p1}; *Ne*/*Ne*. Selection 32-13 traces back to a cross involving the variety

Burbank Preserving, which in turn comes from a cross between *L. pimpinellifolium* and *L. esculentum*: the gene *Ne* was accidentally retained in the selections resulting in the production of 32-13. Burbank Preserving is susceptible to *C. fulvum*; thus *Ne* was retained in the absence of Cf_{p_1}, which must be present if one wishes to test for the presence of *Ne*. This is the only unusual instance in which the factor *Ne* has been encountered.

The Linkage Relations of the Necrosis Factor, *ne*

The linkage tests applied to the factor pair *Ne–ne* involved 16 pairs of contrasting characters. The difference between the members of each pair is controlled by a single pair of alleles. These factors and the corresponding characters are listed on p. 39. In each original cross all the dominant genes under consideration came from one parent and the recessives from the other. Apart from 93 F_2 plants from a cross between *L. pimpinellifolium* and *L. esculentum* and 99 plants from a backcross of such a cross to the esculentum parent, the inheritance of the plants furnishing linkage data was predominantly from *L. esculentum*.

Table VII summarizes the linkage relations of *ne*. The factors d_1, *p*, *o*, and *s* have been located by MacArthur (17) on Chromosome I in the order stated. Our data show that *ne* is located on Chromosome I near *s* on the side distal from d_1, *p*, and *o*. The author feels that the crossover value of 8.1% for *s–ne* may not be as close to the true value as the calculated standard error, 0.7%, suggests. Examination of the backcross data for *S–s* in Table VII reveals a ratio of 820 *S* : 553 *s* and a ratio of 883 *Ne* : 490 *ne*, in the face of expected ratios of 1 : 1 in each case. These deficiencies in the recessives might be caused by one or more of a number of factors, including (1) faulty breeding technique, (2) reduced viability of the recessives, (3) lack of suitable environmental conditions for the development of necrosis or of compound inflorescence in some plants, and (4) combinations of genes, other than *Ne*, that prevent an individual of genotype *ne/ne* from developing necrosis. If some seeds resulted from self-pollination rather than from a backcross to the double recessive decreased numbers of both *s* and *ne* individuals would be expected. Our experience with numerous other populations suggests, however, that this is not likely the explanation. The segregation ratio of 1407 Cf_{p_1} : 1326 cf_{p_1} ($\chi^2 = 2.400$ and $P = 0.12$) in this same population, although not as good a fit to a 1 : 1 ratio as is usually encountered, strengthens this view and indicates that little if any of the deviation in question is due to faulty breeding technique.

Data on the percentage of seed germination in this population were not kept but the behavior of other large populations, grown under environmental conditions favoring the development of necrosis, indicates that we are not dealing with a viability effect here.

The large population used to test linkage among d_1, *p*, *o*, *s*, and *ne* was grown in three sections from seed sown on June 24, a little late for the best field development of necrosis at Vineland Station. Lot 1, of 426 plants, was grown in the greenhouse; Lot 2, of 600 plants, as a staked, field population; and Lot 3,

TABLE VII

LINKAGE RELATIONS OF *ne*, THE NECROSIS FACTOR FROM *L. esculentum*, BASED ON COUPLING DATA ONLY

Factor pair tested and number of chromosome bearing it			n	Segregation ratios[a]				χ^2[b]	Percentage recombination and standard error	Deviation divided by standard error
				XY	*Xy*	*xY*	*xy*			
F_2 data										
D_1	d_1	I	1096	712	138	143	103	73.11	32.7 ± 1.8	9.6
R	*r*	II	934	560	173	149	52	0.44	48.3 ± 2.4	0.7
Y	*y*	III	2008	1095	416	380	117	3.05	53.0 ± 1.7	1.8
C	*c*	IV[c]	1096	675	175	196	50	0.01	50.2 ± 2.3	0.1
A_1	a_1	V	1096	650	200	182	64	0.65	48.1 ± 2.2	0.9
J	*j*	V	93	61	15	12	5	0.77	42.7 ± 7.1	1.0
Lf	*lf*	V	93	61	15	12	5	0.77	42.7 ± 7.1	1.0
L	*l*	VI	1096	701	149	201	45	0.08	49.3 ± 2.2	0.3
U	*u*	VII	1074	587	191	234	62	1.55	52.9 ± 2.3	1.3
H	*h*	VII	1248	758	157	257	76	5.16	45.0 ± 2.0[d]	2.5
T	*t*	VII	1074	580	198	220	76	0.01	49.8 ± 2.3	0.1
Wt	*wt*	X	93	55	21	12	5	0.02	48.8 ± 7.7	0.2
Backcross data *										
D_1	d_1	I	1946	809	381	264	492	204.4	33.2 ± 1.1	15.3
P	*p*	I	987	389	209	109	280	129.2	32.2 ± 1.5	11.9
O	*o*	I	987	391	207	82	307	185.1	29.3 ± 1.4	14.8
S	*s*	I	1373	796	87	24	466	952.4	8.1 ± 0.7	59.9
Y	*y*	III	99	15	31	25	28	2.17	56.6 ± 5.0	1.3
C	*c*	IV[c]	573	146	161	130	136	0.10	50.8 ± 2.1	0.4
A_1	a_1	V	573	159	148	141	125	0.09	50.4 ± 2.1	0.2
L	*l*	VI	573	163	144	152	114	0.94	51.1 ± 2.1	0.5
U	*u*	VII	99	21	25	32	21	2.21	57.6 ± 5.0	1.5
H	*h*	VII	99	20	26	27	26	0.55	53.5 ± 5.0	0.7
T	*t*	VII	99	17	29	19	34	0.01	48.5 ± 5.0	1.7

[a] *X = Ne; x = ne; Y = the dominant and y = the recessive of the factor pair shown in Column 1.*

[b] *The χ^2's of this column, testing independence of X and Y, are determined from 2 × 2 tables.*

[c] *Data showing the independent assortment of Ne and Cf_{p_1}, the latter of which is situated on Chromosome IV, are given in Tables II and III.*

[d] *A more accurate treatment of ne–h data appears on page 54, where the conventional treatment of the hairy stem character as due to a recessive factor has not been followed.*

* *Backcross data for Y, U, H, and T, as well as all the F_2 data, are derived from field populations of 1940, with this exception, that part of the population furnishing backcross data for D, P, O, and S, was grown in the greenhouse.*

the remainder, including the laggards of the population, was grown in the field, untrained. Necrosis developed most extensively and characteristically in the greenhouse lot and least extensively in Lot 3. Inasmuch as Lots 1 and 2 each arbitrarily contained half tall and half dwarf plants and thus were not randomly selected, whereas the residual Lot 3 had a great excess of tall plants,

and inasmuch as *ne* proved to be linked with d_1, *p*, *o*, and *s*, it was decided to combine the data from the three lots. Lot 1 could scarcely be considered an unbiased sample of the population. Nevertheless its crossover value for *ne–s*, $5.9 \pm 0.9\%$, may well be closer to the true value than is $8.1 \pm 0.7\%$. In view of the fairly obvious influence of the environmental conditions in disturbing the ratios it was felt that the most reliable figure for *ne–s* linkage obtainable from the population would be that derived from noting the easily determined *S–s* ratio among plants classified as necrotic. The 490 necrotic plants of this population were classified as 24 with simple inflorescence (*S*) and 466 with compound inflorescence (*s*), yielding a recombination value of $4.9 \pm 1.0\%$. Even this figure is probably too high: the incomplete penetrance of *s* (80.6%) suggests that some individuals of genotype *ne/ne*; *s/s* may appear to be *ne/ne*; *S/?*, but our experiments do not suggest any explanation of the observed deficiency of *s* plants in this population.

The recombination value of $45.0 \pm 2.0\%$, from an F_2 population segregating with respect to the alleles at the *ne* and *h* loci (Table VII), suggests a significant deviation from the figures expected in the absence of linkage. Since, however, the published order of the genes of linkage group VII is *u*, *h*, *t* (17), since this order is confirmed by the populations summarized in Table VII, since the figures for the combinations *ne–u* and *ne–t* show no suggestion of linkage, and since *ne* and *h* show a recombination value of 53.5% in the backcross population, there thus remains no suggestion of a linkage between *ne* and *h*, nor of linkage groups I and VII belonging to a single chromosome. An explanation of the apparent lack of independence of *Ne–ne* and *H–h*, as indicated by both χ^2 and recombination values from F_2 populations, must be sought elsewhere.

A different treatment of the *ne–h* data shows that the lack of independence of the effects of the alleles at or near the *ne* and *h* loci is not as great as the F_2 figures of Table VII indicate. In the preparation of Table VII the conventional treatment of the smooth stem factor as a dominant (17) has been followed. Apparently because of the existence of modifying factors, the expression of the heterozygote, *H/h*, ranges from a moderately hairy condition to a condition in which there are very few long hairs on the plant, but under favorable conditions *H/H*, *H/h*, and *h/h* individuals may be distinguished fairly readily. In the largest of the three F_2 populations providing the 1248 plants used in the calculations reported in Table VII, the *H/H* plants were readily identified but the phenotypes of *H/h* and *h/h* plants were not always distinguishable with certainty. It was felt, therefore, that the classification resulting when the hair condition was considered dominant was more reliable. On the assumption of dominance of the gene for hairiness the *ne–h* F_2 figures of Table VII may be replaced by the following : 569 non-necrotic, hairy; 209 non-necrotic, smooth; 234 necrotic, hairy; 67 necrotic, smooth. The χ^2 value, calculated from a 2×2 table is 2.41 ($P = 0.12$) and the recombination value $46.5 \pm 2.4\%$. These data strengthen the conclusion that there is no linkage between *ne* and *h*.

Unusual Deviations from Theoretical Simple Mendelian Ratios

The inheritance of necrosis, as outlined above (p. 41ff), was apparently so clearly established that the author, primarily concerned at the time with linkage studies and with the development of the variety Vetomold, failed to attach any significance to the occurrence of occasional populations in which the proportion of plants developing necrosis was much below expectation. This past spring, when preparing to summarize all data for publication, it was noted that six such populations, each carrying approximately a 3% inheritance from *L. pimpinellifolium*, also carried the pimpinellifolium factor *H* (smooth stem), which had been selected through four generations of back-crossing as a prospective marker for a new, Vetomold-like tomato. Inasmuch as these six populations were grown in two widely separated locations in the 1938 field plantation, under conditions giving the best field development of necrosis seen during the course of the investigation, with ratios in numerous lines closely approaching the theoretical values (See Tables III, IV, and the adjacent text), an investigation of other genetically similar populations was indicated.

Table VIII is a collection of the data from all populations, other than F_2 populations from crosses between *L. pimpinellifolium* and *L. esculentum*, resulting from the selfing of individuals carrying the factor *H*, usually in the heterozygous condition, and grown concurrently with other populations in which necrosis developed approximately in accordance with the expected frequency. It may be noted that in all but one small population ($3P^s$) the number of necrotic plants is below expectation, that in six of the 17 families the value of *P* is less than 0.05, that the homogeneity of the 17 populations is great, and that the pooled ratio shows an exceedingly wide deviation from a 3 : 1 ratio.

Table IX similarly assembles data from F_2 populations resulting from crosses between *L. pimpinellifolium* and *L. esculentum*. Although the pooled data for the 17 populations show a reasonable approach to a 3 : 1 ratio the heterogeneity of the populations is very high and the data accordingly suspect. It is obvious that the excess of necrotic plants in the large Tangerine F_2 population nearly balances the highly significant deficiency that appears on the pooling of the remaining 16 populations, as shown at the bottom of the table. The homogeneity of these 16 populations is reasonably good. Thus, with the notable exception of the Tangerine F_2 population, the data indicate that populations derived from the selfing of a hybrid between *L. pimpinellifolium* and *L. esculentum*, as well as those from the selfing of other hybrids of genotype Cf_{p_1}/cf_{p_1}; *Ne/ne*; *H/h* (Table VIII), show a great deficiency from the number of necrotic individuals expected.

The classification of F_2 populations from crosses between the two species in question has frequently proved difficult, not only with respect to necrosis, but with respect to segregations involving *p*, *f*, *h*, and *wt*, at least. The behavior of such populations indicates the presence of modifying factors. In the case of necrosis the most sharply defined segregations have been

TABLE VIII

THE INHERITANCE OF NECROSIS IN SEGREGATING TOMATO FAMILIES DERIVED FROM THE SELFING OF INDIVIDUALS OF GENOTYPE Cf_{p_1}/cf_{p_1}; Ne/ne,[d] AND CARRYING THE FACTOR H
(NOTE: FOOTNOTES [a], [b], [c], OF TABLE II APPLY ALSO TO THIS TABLE)

Year and crop	Family designation	Numbers of immune plants			χ^2 (3 : 1)	P (approx.)
		Total	Non-necrotic	Necrotic		
1938, field	2P[a]	25	21	4	1.080	0.3
1938, field	2P[a]	32	28	4	2.666	0.1
1938, field	3P[a]	15	11	4	0.022	0.9
1938, field	4P[a]	33	27	6	0.818	0.4
1938, field	5P[a]	50	47	3	9.627	<0.01[c]
1938, field	5P[a]	20	19	1	4.266	0.04[c]
1938, field	5P[a]	32	29	3	4.167	0.04[c]
1938, field	5P[a]	33	26	7	0.253	0.6
1938, field	5P[a]	18	17	1	3.630	0.05
1939, spring	7P[a]	21	19	2	2.683	0.1
1939, spring	7P[a]	22	19	3	1.515	0.2
1939, spring	7P[a]	21	19	2	2.683	0.1
1939, spring	7P[a]	18	18	0	6.000	0.01[c]
1939, fall	7P[2a]	27	25	2	4.457	0.04[c]
1939, fall	7P[2a]	27[c]	24	3	2.777	0.1
1939, fall	8P[a]	26	24	2	4.155	0.04[c]
1939, fall	8P[a]	27	22	5	0.605	0.4
					51.404	
		447	395	52	42.600	<0.01[c]
	Heterogeneity χ^2 (16 degrees of freedom)				8.804	0.95

[d] *In the case of the third last population only, the plant selfed was of genotype* Cf_{p_1}/Cf_{p_1}.

observed in populations resulting after a number of successive backcrosses to esculentum varieties. Here the necrotic individuals are all essentially alike. In F_2 populations of crosses between *L. pimpinellifolium* and *L. esculentum*, however, considerable variation in the degree of development of necrosis has been observed. The 162 plants classified as necrotic at the bottom of Table IX for example, include 27 that did not show striking and typical symptoms of necrosis although they had time to develop such symptoms. Of these, 11 were classified as moderately necrotic, six as slightly necrotic, and one as very slightly necrotic; varying degrees of doubt were expressed regarding the occurrence of necrosis in the remaining nine plants. These 27 plants were rated as necrotic in the present calculation to avoid any suggestion of bias in favor of the hypothesis advanced to explain the results. This variation in the degree of the development of necrosis, when contrasted with the sharply defined segregations in populations whose inheritance is predominantly from *L. esculentum*, indicates the presence, in *L. pimpinellifolium*, of modifying factors, whose effect is the amelioration of the expression of the gene combination Cf_{p_1}, *ne*.

TABLE IX

THE INHERITANCE OF NECROSIS IN F_2 TOMATO FAMILIES FROM CROSSES BETWEEN *L. pimpinellifolium* AND VARIETIES OF *L. esculentum*
(NOTE: FOOTNOTE [c] OF TABLE II APPLIES TO THIS TABLE)

Year and crop	Esculentum parent	Numbers of immune plants			χ^2 (3 : 1)	P (approx.)
		Total	Non-necrotic	Necrotic		
1938, field	Acme	46	31	15	1.420	0.2
1938, field	Banalbufar	43	33	10	0.070	0.8
1938, field	Beefheart	34	27	7	0.353	0.5
1938, field	Bonny Best	50	43	7	3.227	0.05
1939, field	Break O'Day	76	61	15	1.123	0.3
1938, field	Crackerjack	44	31	13	0.485	0.5
1938, field	Earliana	42	36	6	2.571	0.1
1938, field	Early Detroit Purple	44	34	10	0.121	0.7
1938, field	First of All	46	38	8	1.420	0.2
1938, field	Grand Rapids	88	75	13	4.909	0.03[c]
1938, field	Imperial Globe	44	39	5	4.364	0.04[c]
1938, field	Potentate	85	78	7	12.742	<0.01[c]
1939, field	Rouge Naine Hative	93	76	17	2.240	0.1
1938, field	Sandblast	44	32	12	0.121	0.7
1939, field	Tangerine	1079	778	301	4.827	0.04[c]
1938, field	Tuckswood Favourite	44	34	10	0.121	0.7
1938, field	Wonder of Italy	44	37	7	1.940	0.2
					42.054	
		1946	1483	463	1.514	0.2
	Heterogeneity χ^2 (16 degrees of freedom)				40.540	<0.01[c]
Totals, less Tangerine F_2					37.277	
		867	705	162	18.440	<0.01[c]
	Heterogeneity χ^2 (15 degrees of freedom)				18.787	0.2

Table X assembles data from 19 backcross populations carrying the factor *H* and drawn from eight different crops. A comparison of Table X with Table II, which assembles all backcross data, including those given in Table X, shows clearly that in backcross populations there is no evidence of a deficiency of necrotic individuals in the presence of the factor *H*.

The available data dealing with the relationship between *ne* and Chromosome VII are admittedly inadequate but they are offered now because of the lack, in the present circumstances, of an opportunity to carry out the necessary progeny tests. The factor *ne* has been located on Chromosome I, while *H* is located on Chromosome VII, between *U* and *T* (17). These circumstances, together with the data of Tables VIII, IX, and X, indicate that there is a failure of the development of necrosis in some immune plants of genotype *ne/ne*, in selfed populations, and that this is due to the factor *H* and (or) one or more genes near it. The fact that many plants known to be of genotype *H/H* develop necrosis suggests that a gene or genes near *H* on Chromosome VII constitute an essential part of the inheritance that modifies the expression of

TABLE X

THE INHERITANCE OF NECROSIS IN SEGREGATING TOMATO FAMILIES DERIVED FROM BACKCROSSING HYBRID IMMUNE, NON-NECROTIC, SMOOTH-STEMMED INDIVIDUALS TO ESCULENTUM VARIETIES, I.E. CROSSES OF THE TYPE Cf_{p_1}/cf_{p_1}; *Ne/ne*; $H/h \times cf_{p_1}/cf_{p_1}$; *ne/ne*; *h/h*

(NOTE: FOOTNOTES [a], [b], [c] OF TABLE II ALSO APPLY TO THIS TABLE)

Year and crop	Family designation	Number of immune plants			χ^2 (3 : 1)	*P* (approx.)
		Total	Non-necrotic	Necrotic		
1936, spring	2P	25	13	12	0.040	0.8
1938, field	2P	5	3	2	0.200	0.6
1938, field	2P	11	9	2	4.456	0.04[c]
1939, field	2T[d]	99	46	53	0.495	0.5
1939, field	2T	29	14	15	0.034	0.85
1938, field	3P	13	6	7	0.077	0.8
1937, spring	4P	4	3	1	1.000	0.3
1938, field	4P	17	5	12	2.883	0.1
1937, fall	5P	18	7	11	0.889	0.3
1938, field	5P	18	7	11	0.889	0.3
1938, spring	6P	7	5	2	1.286	0.3
1938, spring	6P	9	6	3	1.000	0.3
1938, field	6P	15	5	10	1.667	0.2
1938, field	6P	15	8	7	0.067	0.8
1938, field	6P	13	9	4	1.923	0.2
1938, fall	7P	18	8	10	0.222	0.6
1938, fall	7P	18	8	10	0.222	0.6
1939, spring	8P	10	4	6	0.400	0.5
1939, fall	8P	18	14	4	5.555	0.02[c]
					23.305	
		362	180	182	0.011	0.9
		Heterogeneity χ^2 (18 degrees of freedom)			23.294	0.2

[d] *The Symbol* T *refers to the variety Tangerine.*

the factor combination Cf_{p_1}, *ne* in a chromosome complex derived predominantly from *L. esculentum*. There has been no suggestion that the factor *H* itself has such an effect.

Discussion

Physiologically, the present studies have not gone beyond the demonstration that light intensity and temperature have a marked effect upon the extent to which autogenous necrosis of tomatoes develops. The leaf symptoms of necrosis, its non-parasitic nature, and the clearly established reduction of its severity through the shading of potentially necrotic plants suggest that the careful study of such physiological characteristics as the transpiration rates of immune non-necrotic and immune necrotic segregants, among the otherwise uniform members of populations resulting from repeated backcrosses to one variety might yield valuable results.

Throughout this article the symbols Cf_{p_1}, cf_{p_1}, *Ne*, and *ne* have been considered to refer to single genes. It is realized, however, that the difference

between immune non-necrotic individuals and immune necrotic individuals may not be due simply to the difference between one pair of alleles. It is quite conceivable, for instance, that the author's *ne* might actually be a very short deficiency in Chromosome I or that his *Ne* might be a very small, localized gene complex. The frequent confusion of gene mutations and deficiencies is discussed in some detail by Goldschmidt (9) in a chapter on the nature of the gene, and in any case is well known to geneticists. Mangelsdorf (18, pp. 161–207), investigating the differences between teosinte and maize through repeated backcrosses to maize, following an original cross between maize and teosinte, has demonstrated segments of chromatin that differentiate teosinte from maize, these segments behaving almost as though they were simple Mendelian factors. In one case, for instance, a backcross resulted in the production of 64 plants with the effect of a certain teosinte segment and 66 plants without these effects. This segment showed linkage with the factor *A* on Chromosome III, and a recombination value of 25%. Other segments broke into smaller fragments, to a certain extent. It is apparent that segment size and frequency of opportunities for crossing over would determine the extent to which the behavior of a chromosome segment might simulate the behavior of a gene. There has been no evidence, in this investigation of necrosis, that we are not dealing with the interaction of the members of two pairs of alleles but these other possibilities are mentioned as a qualification of the author's statement of the simplicity of the inheritance of the normally encountered difference between necrotic and non-necrotic plants immune from *C. fulvum*.

The genetic investigation of necrosis has yielded results of both practical and theoretical importance. In 1942 the author (16) stated that "The Vetomold tomato is essentially the esculentum variety Potentate except for two dominant genes derived from *Lycopersicon pimpinellifolium:* Cf_{p_1}, conferring immunity from *Cladosporium fulvum* strains 1 to 4; and *Ne*, conditioning the compatibility of Cf_{p_1} with esculentum chromosome complexes". The contrasting incompatibility, of which the development of symptoms of necrosis is the visible expression, has been found in the combination of the genotype Cf_{p_1}/?; *ne*/*ne* with a chromosome complex derived largely from *L. esculentum*, whereas the combination of the genotype Cf_{p_1}/?; *Ne*/? with an esculentum chromosome complex results in the production of non-necrotic individuals. The incompatibility appears to be between Cf_{p_1} and an esculentum chromosome complex and not between extensive portions of the chromosome complexes of *L. pimpinellifolium* and *L. esculentum*. It is well known that these two species hybridize readily, producing highly fertile hybrids and there is no suggestion of the type of incompatibility, encountered in many interspecific plant hybrids, which results in partial or complete sterility. No plant of genotype cf_{p_1}/cf_{p_1} has developed necrosis. Possibly the combination of the genotype Cf_{p_1}/?; *ne*/*ne* with a pimpinellifolium chromosome complex would also cause the production of necrosis, but time and space have not been available for the development of this genotype.

The cause of the reduction in the proportions of necrotic plants in populations resulting from the selfing of heterozygous immune, heterozygous non-necrotic plants, bearing the factor *H* (smooth stem) is not clear. The most striking feature of these tests involving *H* is that backcrosses to *L. esculentum* yielded results in accordance with the expectation of 1 immune non-necrotic: 1 immune necrotic while populations resulting from selfing gave significantly less than $\frac{1}{4}$ necrotic individuals among the immune segregants. Among those populations whose inheritance was derived predominantly from *L. esculentum* the populations from selfing would contain individuals homozygous with respect to Cf_{p_1}, *H*, and a few chromosome segments derived from *L. pimpinellifolium* and located adjacent to the loci of Cf_{p_1} and *H* on Chromosomes IV and VII, respectively, while individuals from backcrosses would lack such homozygosity: otherwise the two types of populations are essentially alike. It is thus indicated that some gene or genes in the homozygous condition (and presumably located in the vicinity of the *H* locus) may prevent the development of necrosis in plants having the gene combination Cf_{p_1}, *ne*. An alternative possibility is that such a gene or genes in the heterozygous condition may prevent the development of necrosis when Cf_{p_1} is in the homozygous condition. Further experiments are required to elucidate the nature of the observed effect.

It is interesting to speculate concerning the evolution of the immunity of *L. pimpinellifolium* from *C. fulvum* and of its freedom from necrosis. A hypothesis that appeals to the author is that an ancestor of our strain of *L. pimpinellifolium*, susceptible to *C. fulvum*, evolved an immunity from certain prevalent races of this fungus, Cf_{p_1} being the key factor conferring this immunity. A second result of the presence of this factor was the development of a certain amount of necrosis, its severity depending on the fluctuating environmental conditions. Various factors, from new or from earlier mutations, which ameliorate the severity of the necrosis, were incorporated into the strain through natural selection and such factors are responsible for the variable development of necrosis, from plant to plant, in an F_2 population from a cross between our strain of *L. pimpinellifolium* and various varieties of *L. esculentum*. Among factors modifying the expression of the factor combination Cf_{p_1}, *ne*, one or a group of a few closely linked factors on Chromosome VII near the *H* locus resulted in the complete suppression of necrosis in some immune plants; the evolution of the single factor, *Ne*, likewise provided for the complete inhibition of necrosis. The factor *Ne* and its recessive allele *ne* govern the simple Mendelian inheritance of necrosis.

There is a temptation to reason that modifiers of the combination Cf_{p_1}, *ne* would evolve after the establishment of this gene combination, and certainly it may be reasoned that modifiers that improve the fitness of a plant and thus have survival value are more likely to be retained after than before the development of the factor or group of factors that they modify. In this connection Harland's (13) experiments with cotton are interesting and instructive. Working with crosses between a 'crinkled dwarf' strain of Sea Island cotton

(*Gossypium barbadense*) and various strains of *G. hirsutum*, Harland was able to demonstrate, in certain strains of *G. hirsutum*, groups of modifying factors, distinct from similar factors in Sea Island cotton, whose effect was to make the crinkled dwarf mutant recessive. As this mutant has occurred repeatedly in Sea Island cotton but has never been observed in *G. hirsutum*, Harland's data show very clearly that groups of modifying factors may be incorporated in the inheritance of a plant quite independently of the factor or factors that they modify. Harland concludes that modifiers of dominance are of advantage to the wild type and are thus selected on their own account. His data thus indicate that it cannot be assumed that the modifying factors of *L. pimpinellifolium* here discussed were incorporated in immune strains of this species after the development of the factor Cf_{p_1}. *Ne*, too, may well have originated before Cf_{p_1}.

Considerable experimentation would be necessary to test the validity of certain points in the hypothesis given. For instance, we have no knowledge of the expression of the gene combination Cf_{p_1}, *ne* in the tropical lowlands of Peru, one of the natural habitats of *L. pimpinellifolium* (19), but our experience suggests that severe necrosis might develop in this habitat. The investigation of the modification of the expression of the factor combination Cf_{p_1}, *ne* by the presumed factor or factors near *H* on Chromosome VII is in its preliminary stages only but could be extended readily. The study of plants with the combination Cf_{p_1}, *ne* in a pimpinellifolium chromosome complex might well throw light on this interesting evolutionary problem but such plants have yet to be developed. All our populations trace from a single collection of *L. pimpinellifolium*. The study of the breeding behavior of additional collections of this and other species of *Lycopersicon* that cross with *L. esculentum* may well be a fruitful one and it will be interesting to see whether either of the gene combinations Cf_{p_1}, ne or cf_{p_1}, *Ne* is discovered, particularly in *L. pimpinellifolium*. Alexander, Lincoln, and Wright's (3) survey of the genus *Lycopersicon* for resistance to organisms parasitic on *L. esculentum* is a step in this direction. Others who are breeding tomatoes for resistance to *C. fulvum* may possibly soon provide us with additional data pertinent to these problems. It is hoped that the hypothesis offered may direct the attention of other workers to some of these problems.

Reference has been made to the brief reports of Guba (12) of a "hereditary breakdown" and of Alexander (1) of "what appeared to be a factor for a partial lethal" among F_2 and later derivatives of crosses between *L. pimpinellifolium* and *L. esculentum*. These conditions are almost certainly identical with our 'necrosis' but our data show that the association of necrosis and immunity is not due to linkage, as Alexander reported, but to a factor interaction. The demonstration that the development of necrosis, a serious abnormality superficially resembling somewhat a disease caused by a parasite, may be controlled by members of two factor pairs only, seems to be without a close parallel in other organisms. The author has not been able to find any reference to an abnormality of this type that has been related clearly to the

effect of precise genes. The closest parallel known to him is that of the inheritance of melanism in the platyfish, *Platypoecilus maculatus*, and the swordtail, *Xiphophorus hellerii*, which has been investigated extensively by Gordon (10) and Gordon and Smith (11). Spotted races of *P. maculatus* owe their spotting to the presence of many macromelanophores (large black pigment cells) in its skin, this condition being controlled by a dominant, sex-linked gene, *Sp*. In crosses between spotted and unspotted varieties of *P. maculatus* no unusual results have been encountered. However, a cross of a spotted *P. maculatus* (*Sp*/*Sp*) with an unspotted variety of the swordtail, *X. hellerii* (*sp*/*sp*) yields F_1 hybrids (*Sp*/*sp*) that show a much greater extension of the black pigment than in *P. maculatus* of genotypes *Sp*/*sp* or *Sp*/*Sp*. Such hybrids, characterized as melanotic, have tumor-like growths due to the greatly accelerated growth of the macromelanophores. When these hybrids are backcrossed to *P. maculatus* (*Sp*/*Sp* or *Sp*/*sp*) the hybrids carrying the factor *Sp* are but slightly melanotic; when they are backcrossed to *X. hellerii* (*sp*/*sp*) the *Sp*/*sp* hybrids develop intensified melanosis. Gordon's data show quite clearly that the unfavorable effects of the gene *Sp* are due to its association with an undetermined number of genes from *Xiphophorus*. Somewhat similarly, the abnormality here designated as necrosis is caused by the pimpinellifolium factor Cf_{p_1}, in association with an undetermined number of genes from *L. esculentum*, including *ne* in the homozygous condition.

The autogenous nature of at least some of the cases of necrosis of the leaves of the Chiefkan and Reliant varieties of wheat and of several hybrid lines of wheat has been reported by Chester (7), although there has been no elucidation of the inheritance of such necrosis. Necrotic leaf spotting, commonly known as 'physiological leaf spot' has been observed in certain varieties of wheat, oats, and barley. Chester found that the leaves of plants of most of 80 hybrid lines of wheat developed small watersoaked spots upon being held overnight at 100% humidity in an inoculation chamber but that in most lines these spots disappeared soon after the chamber was aerated in the morning. In other lines the spots remained and became necrotic. In the field only the replicas of these latter lines, and such varieties as Chiefkan, developed 'physiological leaf spot'. Chester concludes that "some wheat strains lack the physiological ability to recover, without injury, from watersoaking due to high humidity, and that this inherited fault is the cause, at least in some cases, of 'physiological leaf spot' ". Chester's data, in conjunction with our own data on environmentally induced variations in the expression of necrosis, suggest the possible fruitfulness of a study of the water relations of potentially necrotic tomato plants. It is of interest also to note that the 80 lines of wheat hybrids investigated by Chester had been developed for resistance to *Puccinia triticina*, and to recall that necrosis in tomatoes occurs only in the presence of an immunity factor Cf_{p_1}. It is quite possible that the spotting in Chester's lines of wheat is one expression of a factor or factors conferring disease resistance.

From 1932 (20) until 1939 the problem of breeding tomatoes for resistance to *Cladosporium fulvum* seemed to be a relatively simple one, except while a method of eliminating necrosis was sought for. The discoveries, from 1939 onwards, of 'new' strains of *C. fulvum* capable of infecting and developing readily upon Vetomold and other similar introductions of the genotypes Cf_{p_1}/Cf_{p_1}; *Ne*/*Ne* (2, 5, 12, 4) has complicated this problem greatly and initiated a search for additional factors conferring resistance to the 'new' strains of *C. fulvum*. Unless there are discovered other factors that confer immunity from *C. fulvum* races 1 to 4, but do not contribute to the development of necrosis, necrosis will continue to be important in breeding programs because of the widespread distribution of races 1 and 3 of the pathogen. Whenever Vetomold or other similar, immune, non-necrotic varieties are crossed with pure esculentum varieties necrosis may be expected among the immune segregants of the second generation. Clarke and Sherrard (8) have already reported such occurrences following crosses with Vetomold. Their 'leaf spot' is undoubtedly our 'necrosis'. The enthusiasm with which Vetomold is regarded, horticulturally, in some localities and its poor performance in others accords with the opinion of many workers that tomato breeding is a somewhat regional problem. The dominance of the two factors conditioning the development of immunity from *C. fulvum* physiologic races 1 to 4 and of non-necrotic foliage makes the backcross method of developing new varieties to suit local conditions a convenient one.

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FURTHER STUDIES ON THE EFFECT OF LEAF RUST ON THE YIELD, GRADE, AND QUALITY OF WHEAT[1]

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Abstract

Experiments were carried out at Winnipeg in 1944, 1945, and 1946 to ascertain the effects of leaf rust (*Puccinia triticina* Erikss.) on the yield, grade, and quality of wheat. Infection in the rusted plots ranged from 22% on some varieties to 87% on others. In these tests, reductions of as much as 26% in yield of straw, 40% in yield of seed, 27% in kernel weight, and 3.5 lb. in bushel weight were caused by infections ranging from 78% to 87%. Lighter infection caused smaller but important reductions. Heavy infection generally reduced grades by one commercial grade while light to moderate infection caused no grade reduction. With but one exception, leaf rust decreased the percentage protein content of the seed, although in the majority of cases it increased baking strength as measured by loaf volume. The carotinoid content of the seed was invariably increased by leaf-rust infection.

Introduction

Leaf rust of wheat (*Puccinia triticina* Erikss.) occurs in Western Canada every year. In some years it spreads through much of the grain growing area of the three Prairie Provinces, and frequently becomes very prevalent in Manitoba and eastern Saskatchewan, where, as has been shown in previous experiments carried out by the authors (5, 6), it not uncommonly causes appreciable crop losses. Since none of the bread wheat varieties presently grown in the rust area of Western Canada possesses much resistance to leaf rust, and the prospects for the early introduction of a highly leaf-rust resistant variety are not very promising, it was decided to continue the investigations initiated in 1938 to determine the effect of leaf rust on the yield and quality of wheat. The results of the experiments carried out in 1944, 1945, and 1946 are given in this paper.

Methods

The experiments were conducted in the field in adequately replicated rod-row plots. One-half of the plots of each variety, in each test, were kept as free from rust as possible by frequent applications of sulphur dust during the growing season. The dust was applied to the plots three times a week at the rate of 30 lb. per acre per dusting from the time rust began to appear in the plots until shortly before the plants matured. The non-dusted plots, in the test of 1944, were artificially inoculated with leaf rust when the plants had reached the fifth-leaf stage. However, in the tests of 1945 and 1946 rust

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inoculum was not artificially introduced into the plots and they became infected by naturally occurring inoculum. The yield of seed, the weight per bushel, the weight per 1000 kernels, and the percentage of protein were determined for each individual plot in each test. In addition, the yield of straw was determined for each plot in the test of 1944. Composite samples of the grain harvested from each lot of plots were graded and tested for milling and baking quality.

The samples were graded by grain inspectors of the Western Grain Inspection Office, Winnipeg, Man. The milling and baking tests were carried out in the Milling and Baking Laboratory of the Cereal Division, Experimental Farms Service, Ottawa. The protein determinations were made in the Chemical Laboratories, Science Service, Ottawa.

Results

Severity of Infection in the Plots

In 1944, leaf-rust infection in the plots became established early and the highly susceptible varieties Saunders and Thatcher were very heavily rusted. The somewhat less susceptible variety, Regent, carried comparatively light infection. In the 1945 test, infections again appeared early and all the varieties tested became heavily rusted. Weather conditions in 1946 were unfavorable for rust development and infections were later in becoming established and were less severe than in the two previous years. Rust was effectively controlled in the dusted plots in all three years and no more than trace infections developed.

Effect on Yield, Kernel Weight, and Bushel Weight

Leaf rust adversely affected the yield of straw of all the varieties in the tests of 1944, the only test for which straw yields were taken, and reduced the yield of seed, kernel weight, and bushel weight of all the varieties in the tests in 1944, 1945, and 1946. In 1944 and 1945, these adverse effects were very pronounced and the reductions were in all cases statistically significant. But in 1946, when rust infections were later in getting established and lighter than in the two previous years the reductions in yield, kernel weight, and bushel weight were small and, with the exception of those for kernel weight, nonsignificant. These findings are substantially in accord with the results of earlier experiments made by the present authors (5, 6) and are in agreement with those of Johnston and Miller (3), Mains (4), and Waldron (7) all of whom found that heavy leaf-rust infection adversely affected yield, kernel weight, and bushel weight. The yield and other relevant data for the experiments made in 1944, 1945, and 1946 are given in Table I.

Effect on Protein Content of the Seed

The percentage of seed protein of the varieties was significantly reduced by leaf rust in both the 1944 and 1945 experiments. In the test of 1946 it reduced the seed protein of Thatcher and Marquis slightly but had no appreciable effect on the seed protein of Redman. Of 36 paired samples from

TABLE I

THE EFFECT OF LEAF RUST ON THE YIELD OF STRAW, YIELD OF SEED, 1000-KERNEL WEIGHT, BUSHEL WEIGHT, AND GRADE OF WHEAT VARIETIES GROWN IN FIELD PLOTS AT WINNIPEG IN 1944, 1945, AND 1946

Variety	Treatment	Amount of infection	Average yield of straw per plot, oz.	Reduction in yield of straw, %	Average yield of seed per acre, bu.	Reduction in yield of seed, %	Average weight per 1000 kernels, gm.	Reduction in kernel weight, %	Average weight per bu., lb.	Grade (Northern)
Experiment of 1944										
Saunders	Dusted	Trace	63.9		52.3		32.3		61.2	2
Saunders	Rusted	78.0	49.1	23.2	32.3	38.2	23.4	27.5	57.5	3
Thatcher	Dusted	Trace	72.5		53.6		30.5		60.0	2
Thatcher	Rusted	87.0	53.5	26.2	31.8	40.7	22.4	26.5	58.5	3
Regent	Dusted	Trace	67.8		50.0		35.5		63.1	2
Regent	Rusted	22.0	60.3	11.1	41.7	16.6	30.1	15.2	61.7	2
Necessary difference[a]			4.9		4.5		1.8		0.9	
Experiment of 1945										
Thatcher	Dusted	Trace	—	—	58.5		27.8		61.4	3
Thatcher	Rusted	82.0	—	—	41.0	29.3	20.4	22.6	59.8	3
Thatcher Selection	Dusted	Trace	—	—	66.4		28.8		62.8	3
Thatcher Selection	Rusted	77.0	—	—	44.9	31.8	21.4	25.7	60.0	3
Hope	Dusted	Trace	—	—	38.9		32.4		59.4	3
Hope	Rusted	52.0	—	—	27.2	30.8	24.6	28.1	57.0	4
Necessary difference[a]					11.3		2.4		1.5	
Experiment of 1946										
Thatcher	Dusted	Trace	—	—	66.5		30.2		63.1	1
Thatcher	Rusted	65.0	—	—	61.1	8.8	26.2	13.3	62.4	2
Redman	Dusted	Trace	—	—	60.6		36.4		62.1	1
Redman	Rusted	45.0	—	—	60.1	0.8	35.2	3.3	62.1	2
Marquis	Dusted	Trace	—	—	55.0		35.2		63.4	1
Marquis	Rusted	55.0	—	—	46.2	16.0	31.4	10.8	63.4	2
Necessary difference[a]					[b]		0.45		[b]	

[a] *Necessary difference, 5% level of significance, between means of dusted and rusted plots within a given variety.*

[b] *Differences between dusted and rusted plots nonsignificant.*

the rusted and nonrusted plots in the 1944 and 1945 tests, the percentage of seed protein in all but one instance was lower for the rusted sample than for the corresponding nonrusted one. Similar results were obtained by the authors (5) in experiments made in 1938, as well as by Caldwell *et al.* (2) in 1930, and by Waldron (7) in 1935 who found that leaf rust reduced seed protein. However, in experiments made by the authors (6) in 1940, samples from plots that had been affected by leaf rust were higher in protein content than corresponding nonrusted samples. Similar results were obtained by Broadfoot (1) in 1927. The data on protein analysis are given in Table II.

Effect on Milling and Baking

Although leaf rust decreased kernel weight and bushel weight it did not cause a parallel reduction in flour yield in so far as experimental milling tests could reveal. Since only one sample was milled from each treatment the experimental error was too large to show small differences.

Leaf rust definitely increased the carotinoid content of the flour and decreased the score for crumb color in the 1944, 1945, and 1946 experiments.

Despite the lower seed protein of the rusted samples the loaf volumes were not lower and in some instances were higher for these samples than for the corresponding non-rusted samples. In all three years mixogram curves on flour–water doughs made from the rusted sample of each variety showed stronger characteristics than those from the corresponding nonrusted sample. Since the percentage of wheat protein was lower for the rusted sample it would be expected that the loaf volume would be lower. But since this was not usually the case, it might be concluded that the quality of the gluten in the flour from the rusted samples was superior. The mixogram curves lend support to that assumption. In experiments made with the 1938, 1940, and 1943 crops it was found that leaf-rust infection did not usually reduce loaf volume; in the majority of instances it improved it. The milling and baking data are presented in Table II.

Effect on Grade

Leaf rust affects grades chiefly by lowering bushel weight. As light leaf-rust infections seldom affect bushel weight appreciably they usually do not have any effect on grade. Heavy leaf-rust infections, on the other hand, particularly when established early, often reduce bushel weights by several pounds and naturally affect grades adversely. Grades, however, are affected by many factors and some of these may obscure or nullify the effects of leaf rust. This happened in the 1945 test when the nonrusted samples, although exceeding the corresponding rusted ones from 1.6 to 2.8 lb. per bushel, were, owing to the presence of green kernels, placed in the same grade as the lightly rusted samples. In the 1944 test the rusted samples of the susceptible varieties were reduced by 3.5 lb. in bushel weight and were reduced in grade by one commercial grade. In 1946 the rusted samples of the three varieties, Thatcher, Redman, and Marquis, graded one commercial grade lower than corresponding nonrusted samples. However, as the rusted samples were

TABLE II

THE EFFECT OF LEAF RUST ON THE FLOUR YIELD, FLOUR COLOR, PERCENTAGE OF SEED PROTEIN, LOAF VOLUME, CRUMB TEXTURE, AND CRUMB COLOR OF WHEAT VARIETIES GROWN IN FIELD PLOTS AT WINNIPEG IN 1944, 1945, and 1946

Variety	Treatment	Rust infection, %	Flour yield, %	Flour color (carotene), p.p.m.	Seed protein, %	Loaf volume, cc.	Crumb texture	Crumb color
Experiment of 1944								
Saunders	Dusted	Trace	76.7	1.6	14.7	913	7.5	—
Saunders	Rusted	78.0	72.6	2.3	13.9	888	7.5	—
Thatcher	Dusted	Trace	72.7	1.3	15.0	870	8.3	—
Thatcher	Rusted	87.0	71.7	2.1	13.5	813	7.5	—
Regent	Dusted	Trace	72.5	1.2	15.8	913	8.5	—
Regent	Rusted	22.0	72.7	1.5	14.7	993	6.5	—
Necessary difference[a]					0.33			
Experiment of 1945								
Thatcher	Dusted	Trace	75.8	1.7	15.5	795	7.5	7.0
Thatcher	Rusted	82.0	75.2	2.3	14.7	885	7.5	6.3
Thatcher Selection	Dusted	Trace	74.8	1.6	15.2	840	8.0	8.5
Thatcher Selection	Rusted	77.0	75.6	2.2	14.3	870	8.0	8.3
Hope	Dusted	Trace	76.0	1.7	16.2	795	8.0	8.5
Hope	Rusted	52.0	75.2	2.3	15.5	830	8.0	8.0
Necessary difference[a]					0.49			
Experiment of 1946								
Thatcher	Dusted	Trace	70.8	1.6	15.1	835	7.8	7.0
Thatcher	Rusted	65.0	72.6	2.0	14.7	885	7.8	6.3
Redman	Dusted	Trace	74.8	1.3	15.2	875	8.0	8.5
Redman	Rusted	45.0	74.6	1.4	15.5	940	8.0	8.3
Marquis	Dusted	Trace	71.9	1.3	16.1	945	8.0	8.5
Marquis	Rusted	55.0	74.1	1.5	15.4	915	8.0	8.0
Necessary difference[a]					[b]			

[a] *Necessary difference, 5% level of significance, between means of dusted and rusted plots within a given variety.*

[b] *Difference between dusted and rusted plots nonsignificant.*

degraded on account of the presence of immature kernels in the sample but not because of reduction in bushel weight or for any other condition attributable to the effect of leaf rust, it must be assumed that the grade reductions of the rusted samples, in that year, were brought about by causes other than leaf rust.

Discussion

These experiments indicate that light leaf-rust infections have little or no effect on grade, but that heavy infections, when other grade influencing factors do not interfere, may cause grade reductions. Leaf rust apparently has a marked effect on the carotinoid content of the grain, for the rusted samples in every test were appreciably higher in yellow pigment than the nonrusted ones. Although the rusted samples were usually, but not always, lower in percentage of protein than the nonrusted samples, they possessed, in the majority of cases, greater baking strength and produced loaves of greater volume than did the nonrusted samples. The rust apparently, in as yet some unexplained manner, favorably modified protein quality.

The effects of leaf rust on kernel weight, bushel weight, and yield were more pronounced than those on quality and grade. Heavy infections invariably caused very substantial kernel weight, bushel weight, and yield reductions, and even a moderately light infection (22%) such as occurred on Regent in the test of 1944, caused appreciable reductions. This demonstrated ability of leaf rust to injure the crop even when present in only moderate amounts suggests that the economic importance of this rust may be underestimated. Small but significant reductions such as those suffered by Regent in the above-mentioned test would certainly remain undetected under ordinary farm practices.

As no approved commercial bread wheat varieties possessing high leaf-rust resistance are now extant it would appear most desirable to make such varieties available to growers at the earliest time possible. The work now in progress with that end in view, therefore, should now receive added emphasis.

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WINTER CROWN ROT OR SNOW MOLD OF ALFALFA, CLOVERS, AND GRASSES IN ALBERTA

I. OCCURRENCE, PARASITISM, AND SPREAD OF THE PATHOGEN[1]

By M. W. Cormack[2]

Abstract

Winter crown rot or snow mold of alfalfa, clovers, and grasses, caused by an unidentified low-temperature basidiomycete, is widespread and often of major importance in the central and northern areas of Alberta and Saskatchewan. For example, during a six year period an average of 62% of the alfalfa stands examined in west-central Alberta were affected, and the average estimated damage was 10%. Severe damage also occurs in alsike and white Dutch clover, and in timothy, red top, and creeping red fescue. The disease is less prevalent in red clover, Kentucky bluegrass, and meadow fescue, and seldom occurs in brome grass, crested wheat grass, and slender wheat grass. Iris and other garden perennials, as well as dandelion, quack grass, and various wild plants are also damaged.

The pathogen attacks the dormant plants beneath the snow during the first thaw in the late winter or early spring. The plants are killed or weakened in irregular patches as the result of rotting of the crown buds and tissues. These symptoms are distinct from those of true winter killing, with which the damage has been confused. The pathogen is difficult to isolate, except at a temperature near freezing from superficial mycelium or freshly infected tissues.

The results of infection experiments in the field and under controlled conditions indicate that the development of the disease is associated with physiological changes in the host. Inoculated alfalfa plants brought inside at weekly intervals became susceptible at dates varying from late November to late December in different seasons. Infection was most severe under the conditions provided by a slowly melting snow cover and was also influenced by soil temperature, soil moisture, and growth of the pathogen.

The pathogen appears to spread mainly by means of mycelium, since no sporulating stage has been found. The mycelium spreads both above and below ground at the time of the first spring thaw. The distance of radial spread, as measured by killing of the plants, varied in different years from 2.7 to 7.5 in. in dense alfalfa stands, and from 0.5 to 6.0 in. in bare land.

Winter crown rot is the name now proposed for a previously undescribed disease of alfalfa and other forage crops that is prevalent and sometimes very destructive in Alberta and Saskatchewan. It is commonly called snow mold in turf grasses (1). This disease, frequently confused with true winter killing, is caused by an unidentified low-temperature basidiomycetous fungus (2) that attacks the dormant plants beneath the snow when thawing first starts in the late winter or early spring. As in the case of *Plenodomus Meliloti*, studied by Sanford (7), the fungus ceases to cause damage before growth is resumed. The pathogen is more virulent and injurious to alfalfa and clovers than any of the root- and crown-rotting fungi previously studied in Alberta (3).

Considerable information has been obtained on winter crown rot during the past seven years, but several phases of the investigation are not yet completed.

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This first paper of a series will deal primarily with the disease symptoms, distribution, parasitism, and manner of spread of the pathogen. The results of field studies on host range, varietal reaction, persistence, and control, and on the cultural characteristics and taxonomy of the pathogen, will be reported later.

Symptoms

The occurrence of dead plants in irregular patches (Figs. 1, 2, 10, 11) is the most characteristic symptom of winter crown rot damage in alfalfa and clover stands in the central and northern areas. These dead patches first become evident in the spring when growth starts in adjacent unaffected areas. They are most common on slopes, but may occur at random in a level field. Scattered individual plants or small patches (Figs. 3, 4) are usually killed first, forming a starting point for larger patches that extend from year to year. Large patches may be killed in a single year if winter or early spring conditions are particularly favorable for the disease. Dandelions or other weeds soon occupy the bare areas (Fig. 5). In southern Alberta the winter crown rot pathogen most commonly causes partial rotting of the crowns and weakening of alfalfa plants, rather than killing in patches as in the central and northern areas. This damage is often not very evident unless the plants are examined closely or dug up.

An alfalfa or clover plant affected by winter crown rot has dark brown rotted areas at the crown, and, less commonly, lower on the root (Figs. 6 to 9). This damage is apparently distinct from that reported by Jones (5) in alfalfa clones in Wisconsin. The extent of the development of lesions varies greatly in different seasons and localities. Plants are often killed or greatly weakened when only the crown buds and a very small portion of the underlying tissues are affected (Fig. 6). At the other extreme, the entire crown and upper portion of the root may be severely rotted (Fig. 7). Even when the plant is killed, the main root usually remains relatively sound until natural decay sets in. When the crown is not completely rotted, some of the buds usually escape and start weak growth late in the spring (Fig. 6). This may result in proliferation of a portion of the crown during the growing season (Fig. 8). Another sign of previous infection is the coarse, whitish mycelial remnants of the pathogen sometimes found on or near the crowns of dead or damaged plants.

Winter crown rot damage is much more common than true winter killing in hardy varieties of alfalfa in Alberta. Winter killing usually occurs only

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Alfalfa naturally infected with the winter crown rot pathogen.

FIGS. 1, 2. *Dead areas in severely damaged stands.* FIG. 3. *Initial killing in small patches.* FIG. 4. *Close view of dead patch.* FIG. 5. *Growth of dandelions and other weeds in dead areas.* FIG. 6. *Plants weakened or killed through rotting of the crown buds and underlying tissues.* FIG. 7. *Severe rotting of the crown and root.* FIG. 8. *Severely weakened old plant with partially proliferated crown.* FIG. 9. *Split root of old plant with completely rotted crown.*

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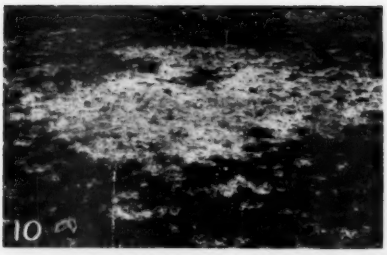
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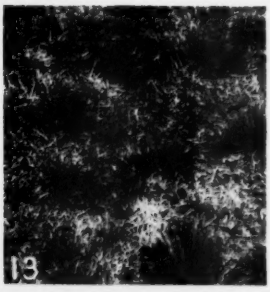
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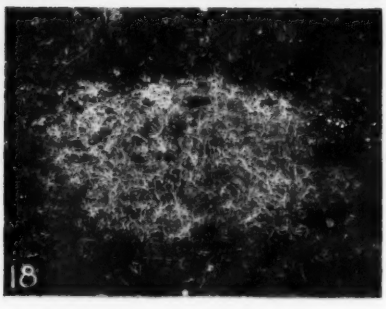
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under exceptionally severe weather conditions when there is an ice sheet or insufficient snow cover, or after the plants have prematurely lost their hardened condition in the early spring. In winter killing all or most of the root system is affected, the root becomes soft and shredded, and the plant is easily pulled up. Plants partially injured as the result of adverse winter conditions have variable symptoms but lack the distinct crown lesions characteristic of winter crown rot (Figs. 6 to 9). Occasionally the symptoms of winter crown rot and true winter killing are found in the same field and even on the same plants.

Forage grasses attacked by the winter crown rot pathogen suffer damage similar to that caused by snow mold in turf grasses, described by Broadfoot (1). As in the cases of alfalfa and clover, the killing of timothy (Figs. 12, 13) creeping red fescue, and red top usually occurs in patches. Similar damage occurs in mixed stands of the legumes and grasses (Figs. 14 to 16). In some seasons extensive killing is also found in native pastures and meadows (Figs. 17, 18). These bare areas eventually fill in with annuals and quack grass or other rhizomatous plants.

Distribution and Importance on Different Hosts

Alfalfa

Winter crown rot has been found in all districts in which alfalfa seed is grown and also in many hay and pasture fields in central and northern Alberta and northern Saskatchewan. Particularly severe damage has occurred in the districts of Cherhill, Sangudo, Thorsby, and Westlock in west-central Alberta, and White Fox in northeastern Saskatchewan. In southern Alberta the disease occurs in many irrigated and nonirrigated stands of alfalfa, but causes relatively slight damage. The pathogen has also been isolated from diseased specimens sent from damaged stands in British Columbia and Manitoba. It has not yet been reported outside of Western Canada.

The severity of winter crown rot damage varies greatly from year to year. In west-central Alberta the general damage was estimated as moderate to severe in four out of six years (Table I). The disease was most prevalent and severe in 1943, when 88% of the stands examined were infected and had an average estimated damage of 20%. Many stands were also ruined in 1941, 1942, and 1946, through killing of from 25 to 50% of the plants. In 1944 and 1945 there was relatively little damage, consisting mainly of a slight increase in size of the previously killed patches, or in the killing or weakening of individual plants or small patches (Fig. 3). This damage, however, undoubtedly helped in the incidence of severe killing again in 1946. During

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Legumes and grasses naturally infected with the winter crown rot pathogen.

FIGS. 10 to 13. *Dead areas in—10: alsike clover; 11: white Dutch clover; 12 and 13: timothy.* FIGS. 14 to 16. *Varying degrees of damage in mixed stands—14: alsike clover and timothy; 15: red top and alfalfa (left), brome grass and alfalfa (right); 16: creeping red fescue and alfalfa.* FIGS. 17, 18. *Dead areas in native pasture.*

TABLE I

ESTIMATED DAMAGE CAUSED BY THE WINTER CROWN ROT PATHOGEN IN ALFALFA STANDS EXAMINED IN WEST-CENTRAL ALBERTA (1941-46)

Year	Percentage of stands[a] damaged					Total stands diseased, %	Average estimated damage in diseased stands, %
	None	Trace	Slight	Moderate	Severe		
1941	15	18	20	20	27	85	16
1942	36	12	18	16	18	64	15
1943	12	10	14	31	33	88	20
1944	67	9	22	2	—	33	3
1945	66	12	19	3	—	34	3
1946	28	3	21	23	25	72	18
Average for six years						62	10

[a] *Average of 120 stands examined each year.*

the six year period the average estimated damage was 10%, with an average of 62% of the stands affected (Table I).

The amount of damage in different districts also varies greatly, apparently as a result of local environmental conditions during the winter. For example, the killing was particularly severe in northeastern Saskatchewan in 1942, and in west-central Alberta in 1943. As previously noted, infection is always less severe in the southern districts, where it results mainly in partial rotting of the crowns, with consequent weakening of the plants. This damage is not as important as the killing that occurs in the central and northern areas, but it probably contributes considerably to reduction in yield, as well as to shortening the life of the crop stands. In the irrigated areas winter crown rot commonly occurs in combination with the bacterial wilt disease.

Winter crown rot is most evident in relatively young, fairly thick alfalfa stands, where the pathogen apparently has the best opportunity to spread from plant to plant. It is usually most severe in stands three to four years old and seldom occurs in one-year-old stands, except in heavily infested land where a diseased crop has been recently ploughed up. The damage becomes less noticeable and may even cease after the plants become thinned out due to winter crown rot or other causes.

Observational evidence indicates that winter crown rot can be important under a wide range of soil conditions. It has been severe in alfalfa growing on both light and heavy soils in the black, transition, and gray wooded soil zones of Alberta. That damage is less in the brown soil zones of the southern districts is apparently explained by climatic conditions. In several instances the disease developed on alfalfa on new breaking and also following cereals and various other crops.

Observations made during the early season on losses due to winter crown rot may be quite misleading, since the damage cannot be accurately estimated

until late May or early June, when general growth is well advanced. Many plants that appear dead early in the spring may start late growth from crown buds that have escaped serious injury (Fig. 6). If the crown tissues are not too severely rotted and growth conditions are favorable, this weak late growth may give a fair yield of hay, especially on the second cut. Such growth is, however, usually too late for satisfactory seed production. Also, the weakened plants often do not survive the following winter.

Clovers

Winter crown rot is frequently destructive in alsike clover, especially in the seed-growing areas of west-central Alberta. For example, it caused moderate to severe damage in 7 out of 17 fields examined in 1946. White or Dutch clover is also very susceptible and sometimes suffers severe damage along roadsides or when grown occasionally as a seed crop (Fig. 11). Vanterpool (9) found that white clover was killed out in snow mold patches in lawns at Saskatoon. Stands of red clover are often less severely affected, since the plants are usually killed individually rather than in patches. Red clover is less hardy than other clovers and alfalfa under Alberta conditions, and disease damage is sometimes confused with winter killing. Sweet clover suffers extremely severe damage when planted in land naturally or artificially infested with the pathogen.

Forage Grasses

The winter crown rot pathogen often causes damage in timothy, red top, and creeping red fescue in the central and northern districts. In 1946 severe damage was found in 9 out of 16 fields of timothy and in several mixed stands of timothy and alsike clover in west-central Alberta. The pathogen has also been isolated from naturally infected plants of meadow fescue and Kentucky bluegrass, but appears to be less prevalent on these hosts. It has seldom been found in brome grass, crested wheat grass, or slender wheat grass. Mixed stands consisting of alfalfa or clovers with susceptible grasses such as timothy, red top, or creeping red fescue usually suffer much more damage than those containing brome grass, which appear to be highly resistant (Figs. 14 to 16).

Other Hosts

The results of isolation and infection studies, not yet completed, indicate that the winter crown rot pathogen may cause damage in a wide range of cultivated and wild perennial plants. It has been isolated from naturally infected plants of the following cultivated species: *Iris germanica* L.; *Aconitum* sp.; *Phlox paniculata* L.; *Thymus Serpyllum* L.; and *Pastinaca sativa* L. Isolates have also been frequently obtained from diseased plants of *Taraxacum officinale* Weber, and *Agropyron repens* (L.) Beauv., and from the mycelium and dead plants of various species in snow mold patches in native pastures and meadows. The fungus was previously isolated by Broadfoot (1) from

severely damaged turf grasses at Edmonton, and by Vanterpool (9) from snow mold patches in lawns at Saskatoon. No damage has yet been found in plants growing in wooded land.

Isolation Studies

Isolation of the winter crown rot pathogen is usually difficult, except from superficial mycelium or freshly infected host tissues obtained during the spring thaw. Later, the soil temperature starts to rise, and the diseased tissues are invaded by secondary parasites and saprophytes that often inhibit or retard growth of the pathogen, especially at temperatures above 10° C. Isolates are most easily obtained by plating fragments of mycelium from the surface of diseased tissues, and incubating them in a refrigerator at about 1° C. Potato dextrose agar is one of the most favorable media for isolation and growth of the pathogen. If superficial mycelium is not present, the fungus usually grows more readily from the base of the stems than from the crown or root tissues of diseased plants. Direct plating of the material without prior washing or surface sterilization generally gives the best results, since mercuric chloride and other fungicides tend to retard growth of the fungus.

Isolates of the pathogen grow slowly from plated mycelium or diseased tissues, and require about 10 to 14 days to produce observable growth on potato dextrose agar at 1° C. Once established, the whitish mycelium grows fairly rapidly in close contact with the medium, forming a zoned matted colony with straight radial growth of the hyphae clearly visible at the edge. White stroma-like structures are sometimes produced but no spores or sclerotia have been observed. The fungus can be identified microscopically by the numerous, typically basidiomycetous, clamp connections fully developed on the septate mycelium in colonies about two weeks old.

Infection Studies

Field Experiments

Field infection of alfalfa and other hosts by the winter crown rot pathogen was studied by planting in infested land and by inoculation. Inoculations were made in the fall by applying inoculum grown on a soil–cornmeal medium or natural inoculum consisting of soil or sods taken from dead patches in fields or meadows. Individual plants were inoculated by placing a pinch of inoculum in the surface soil near the crowns and a similar method was used to start infection at marked points in rows or plots. In large plots or blocks of plants the inoculum was broadcast or spread thinly in a shallow trench near the rows. Since very extensive killing usually resulted from inoculation by the above method, many experiments were conducted in naturally and artificially infested soil.

Final notes on infection were taken after growth of the healthy plants was well started in the spring. In some experiments the dead plants were counted, and in others, the distance of spread of the killing in plots or rows was

measured. Plants that were damaged but not killed were given an infection rating based on the extent of rotting of the crown buds and tissues.

The crown and root symptoms resulting from inoculation or from planting in infested soil were similar to those observed under natural conditions (Figs. 6 to 9). Mycelial growth of the pathogen, however, was much more evident. It remained around the crowns of diseased alfalfa plants (Fig. 19) for a few days after the snow melted. This grayish-white, cobweb-like growth sometimes extended up the dead stems of the affected plants (Figs. 20, 21). In grasses and clovers the mycelium formed snow mold patches (Figs. 25, 26) similar to those found in turf grasses. The plants beneath the mycelium were usually killed. Large blocks of alfalfa were completely killed (Fig. 22) by inoculation or by planting in heavily infested land. When inoculum was placed at staked positions in the plots the plants were killed in patches (Fig. 24) similar to those occurring in naturally infested fields (Fig. 4). These patches enlarged and coalesced during successive years (Fig. 23).

Crock Experiments

Since it was not possible to make accurate observations on the early stages of field infection, attempts were made to study the disease under controlled conditions. Field-grown alfalfa plants were transplanted into 1-gal. crocks of soil in late October. In most experiments, the crocks, each containing 10 plants, were set in a shallow pit outside the greenhouse, with the rims at ground level and the intervening spaces filled with soil or peat.

Beginning in early November, two crocks of inoculated plants and a control were taken in at weekly intervals to study the progress of infection under various controlled conditions. These crocks were kept for a week at low temperatures to allow disease development and were then placed in the greenhouse. Final notes were taken when growth was well advanced in the control plants.

Factors Influencing Disease Development

Attempts to explain the varying severity of winter crown rot damage from year to year and in different localities solely on the basis of general winter conditions have not been very successful. For example, severe damage occurred in many alfalfa fields in west-central Alberta in 1941, 1943, and 1946 (Table I), following winters that differed markedly in average air and soil temperature, snowfall, and other conditions. Other factors involving the pathogen and the host apparently interact with environmental conditions in influencing disease development in the late winter or early spring.

Growth of Inoculum

The results of inoculation studies indicate that disease development depends to some extent upon previous growth of the mycelium of the pathogen. In crock experiments, freshly grown inoculum placed in contact with susceptible plants required at least two weeks of undisturbed growth under favorable

conditions before it would cause infection. For example, in the 1944-45 experiment, infection of plants inoculated at various times and thawed at 2° C. in January was as follows:

Inoculated on Oct. 10.....	100%
Inoculated on Nov. 7.....	87%
Inoculated on Dec. 5.....	13%

Late inoculation apparently did not allow sufficient time for growth of the inoculum before freeze-up, since much more mycelium developed about the crowns of the plants inoculated in October than in December.

In field experiments, October inoculations invariably resulted in a higher degree of infection the following spring than those made after freeze-up in late November. No infection occurred when inoculations were made in the late winter or early spring. As in naturally infested soil, fall inoculation apparently affords the pathogen an opportunity to develop before conditions are optimum for infection in the late winter or early spring.

Dormancy of the Host

Under field conditions, winter crown rot damage always ceases while the snow is melting. Also, all attempts to secure infection of growing plants have failed. Evidence that the low-temperature basidiomycete is strictly a parasite of the dormant plants was confirmed in crock experiments conducted during seven winters, from 1940 to 1947, inclusive. Crocks of inoculated and control plants, removed from an outside pit at weekly intervals starting in early November, were held at about 2° C. for a week. No infection occurred in the first series of plants, even when there was an abundant growth of mycelium around the crowns. In subsequent series, disease development was at first restricted to the rotting of a few crown buds on each plant. As the season advanced the plants became progressively more susceptible (Table II and Figs. 27 to 30) until finally they were killed or greatly weakened (Fig. 30). The time when they first became susceptible to attack and the rate of increase in disease development varied considerably from year to year (Table II). The earliest and most rapid development occurred during 1943 to 1944, when the plants were first attacked in the series brought in on Nov. 17, and were completely susceptible in the Dec. 8 series. In contrast, during the winter of

Winter crown rot development resulting from inoculation.

Fig. 19. *Mycelium of the pathogen in rows of dead alfalfa in early March.* Fig. 20. *Mycelium on alfalfa kept free of snow (top rows), and with normal snow cover (bottom row).* Fig. 21. *Mycelium on tops of dead alfalfa plants.* Fig. 22. *Plot of alfalfa completely killed by pathogen compared to noninoculated control plot at left.* Figs. 23 to 26. *Dead patches produced as a result of placing inoculum at staked positions in the fall—23: extensive killing of alfalfa in successive seasons (below), two years after inoculation of healthy stand (above); 24: close view of artificially produced dead patch in alfalfa; 25, 26: mycelial patches in rows of clover in early March.* Figs. 27 to 30. *Crocks of alfalfa plants brought inside and thawed at intervals in 1946-47—27, 28: Dec. 2 series, control and inoculated, respectively; 29: Dec. 16 series, inoculated; 30: Jan. 6 series, inoculated.*

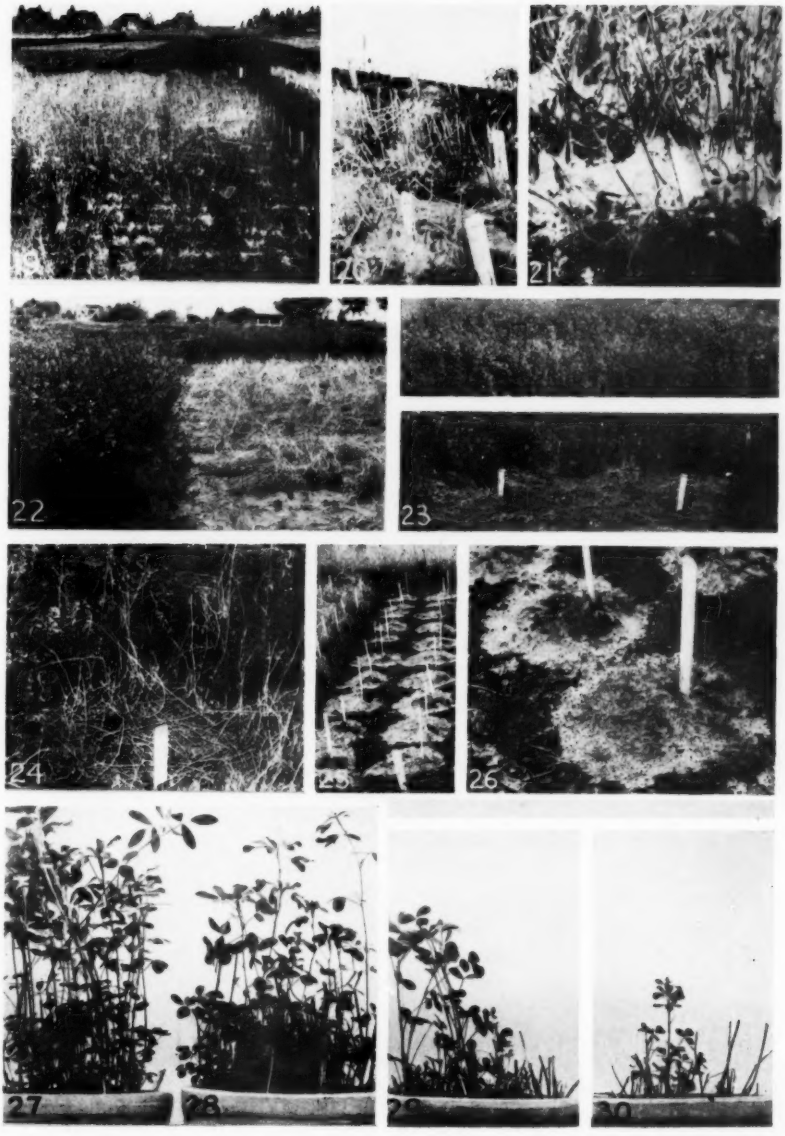
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TABLE II

RELATION OF SOIL TEMPERATURE AND SNOW COVER TO THE TIME AND EXTENT OF WINTER CROWN ROT DEVELOPMENT IN INOCULATED ALFALFA PLANTS BROUGHT IN AT WEEKLY INTERVALS DURING THE EARLY WINTER (1941-47)

Winter	Disease development			Av. soil temp.[a], ° F.		Snow cover	
	First	Moderate	Severe	Nov.	Dec.	Nov.	Dec.
1943-44	Nov. 17	Nov. 24	Dec. 8	30.4	30.2	None	Light
1945-46	Nov. 26	Dec. 3	Dec. 10	23.7	21.3	Light	Light
1940-41	Nov. 25	Dec. 9	Jan. 3	29.2	28.0	Light	Light
1941-42	Dec. 1	Dec. 15	Jan. 5	30.8	21.1	Light	Light
1946-47	Dec. 2	Dec. 16	Jan. 6	27.7	22.7	Light	Moderate
1942-43	Dec. 7	Dec. 21	Jan. 4	29.6	28.6	Moderate	Heavy
1944-45	Dec. 11	Dec. 26	Jan. 9	22.5	17.2	Moderate	Moderate

[a] *Recorded at a depth of 3 in.*

1944 to 1945 no infection occurred in any series until Dec. 11, and the damage was not severe until Jan. 9. These results indicate that susceptibility to attack by the pathogen is associated with the physiological changes accompanying dormancy and hardening in the host plant, and that the rate of these changes varies from year to year.

Soil Temperature

Observations indicate that soil temperature does not have a direct effect on the degree of winter crown rot development. Severe damage occurred in 1942 and 1946 (Table I), when the average soil temperature during the winter was low (about 20° F.), and in 1941 and 1943, when it was relatively high (25° to 30° F.). In 1945 the damage was only slight after a winter during which the soil temperature dropped to 10° F.

Soil temperature appears to affect the development of root lesions. When the average soil temperature was relatively high and there was little frost in the ground, the root lesions often appeared for some distance below the crowns (Fig. 7). On the other hand, the lesions were usually confined to the crowns (Fig. 6) following winters when the ground was frozen hard.

The results of crock experiments (Table II) indicate that the time and rate at which the plants become susceptible to attack are not directly related to low soil temperatures. The average soil temperature was about 30° F. during November and December of 1943 to 1944, when the plants became most rapidly susceptible. Also, in other years there was no apparent correlation between soil temperatures and changes in host susceptibility. Freezing temperatures, however, proved necessary to bring about these changes. In several experiments, the plants in crocks placed close to the greenhouse, where radiation of heat delayed freezing, did not become susceptible so early as, and they suffered much less damage than, the plants in normally frozen soil.

Soil Moisture

The influence of soil moisture on disease development was studied by adding varying amounts of water to the soil in a series of crocks of inoculated plants. The moisture content of the soil could not be accurately controlled in the outside pit, but water was added when necessary to maintain the gradient between the different series. The average degree of infection obtained in a representative experiment (1944 to 1945) was as follows:

Dry soil series (about 35% m.h.c.)	77%
Moist soil series (about 50% m.h.c.)	60%
Wet soil series (about 70% m.h.c.)	28%
Saturated soil series (about 90% m.h.c.)	None

In all crock experiments there was decidedly less infection of the plants in wet or saturated soil than of those in dry or moist soil. Although the differences were less marked under field conditions, disease development was usually less severe in plots watered heavily in the late fall than in those not watered.

Snow Cover

Observations made over a period of years indicate that the amount of snow cover has much less influence on the development of winter crown rot than the time and manner in which it melts. The snow cover was variable in 1941, 1943, and 1946, when the most severe damage occurred (Table I), but in all three years it melted slowly, starting in late March or April. Winter crown rot damage in the experimental plots was also particularly severe in these three years and it was associated with an especially profuse growth of mycelium (Figs. 19 to 21). There was much less damage and also less mycelial growth in years when the snow melted early and rapidly. A similar relationship was observed in field experiments where one set of plots was completely cleared of snow prior to thawing in the early spring. A second set of plots was partially cleared at intervals during the winter, and a control set was left with an undisturbed snow cover. The average radial spread of the killing in these plots for a period of three years (1943 to 1946) was as follows:

Snow cleared before thawing	2.0 in.
Light snow cover	2.4 in.
Undisturbed snow cover	3.2 in.

Early removal of the snow apparently prevented maximum development of the pathogen, since there was much less mycelial growth in the cleared plots than in those left undisturbed.

The effect of temperature on the rate of thawing and disease development was studied in crock experiments. Crocks containing plants that had reached a susceptible stage were removed in late December from the outside pit described above and were thawed at controlled temperatures ranging from 1° to 20° C. They were then placed in the greenhouse until growth started

in the control plants. The average degree of infection obtained in a representative experiment (1945-46) was as follows:

Thawed in four days at 1° C.	73%
Thawed in two days at 5° C.	41%
Thawed in one day at 9° C.	12%
Thawed in 12 hr. at 15° C.	11%
Thawed in six hours at 20° C.	None

In all experiments, slow thawing at a temperature near freezing favored disease development, regardless of the presence or amount of snow on the crocks.

Field studies also indicated that snow in itself was not essential for disease development, but merely provided favorable conditions. Replicated rows of inoculated alfalfa plants were kept free of snow throughout the winter by covering them with V-shaped wooden troughs with closed ends. These troughs were left on the rows until the snow melted in adjacent rows. Infection was as severe under the troughs as in the rows with a normal snow cover, and mycelium was abundant in both cases (Fig. 20).

The influence of snow cover on host dormancy and susceptibility in the late fall was also studied. As shown in Table II, the earliest and most rapid disease development occurred in years when there was little or no snow cover during November and December. In other winters the insulating effect of a heavier snowfall may have retarded the action of low temperatures on the physiological changes in the plants.

Presence of Other Pathogens

The association effects of other root and crown rotting fungi frequently isolated from plants primarily parasitized by the low-temperature basidiomycete were studied. In crock experiments, inoculum of the latter was mixed in equal proportions with that of each of several other pathogens, including *Cylindrocarpon Ehrenbergi* (4), *Plenodomus Meliloti* (7), *Sclerotinia sativa*, and *Fusarium avenaceum* (3). Complete killing of the plants occurred in all tests, but the presence of other pathogens, especially *C. Ehrenbergi* and *S. sativa*, resulted in more extensive rotting of the roots below the crown than that caused by the low-temperature basidiomycete alone. These results support the observation that certain root-rotting pathogens may sometimes increase the damage started by the winter crown rot pathogen.

Field Spread of the Pathogen

Manner of Spread

Aside from possible mechanical transfer by implements or other means, the winter crown rot pathogen appears to spread mainly by means of mycelium. No fruiting stage that would aid in its spread has yet been found in nature or

in artificial culture. Also, the extent and manner of spread of the fungus in the field do not indicate that spores are involved. For example, it did not spread across a 2 ft. path from infested experimental plots to control plots (Fig. 22), when reasonable precautions were taken.

It has been apparent that the fungus might spread both above and below ground, since infection was severe when the mycelium was abundant around the crowns (Figs. 19 to 21), and also sometimes when it was scarce or absent. In field experiments with alfalfa, various barriers were used in an attempt to determine the relative importance of these two types of spread. Replicated 6 in. pots, placed at intervals along the rows of plants, proved most suitable. For the aboveground series, inoculum was placed on the inside edge of the rim of pots nearly filled with soil. Each pot was buried so that the rim was at soil level and the pathogen had to pass over it and the intervening ground to reach the plants. To force spreading of the mycelium below ground, empty pots were inverted and inoculum was placed inside the slightly buried rims. In both series, three sets of pots were placed so that the inoculum was at distances of one-half, one, and two inches, respectively, from the crowns of the plants. Notes were taken in the spring on the extent of the killing in the row opposite each pot.

TABLE III

RELATIVE DEGREE OF KILLING OF ALFALFA PLANTS BY THE WINTER CROWN ROT PATHOGEN WHEN INOCULUM WAS PLACED IN DIFFERENT POSITIONS ABOVE- AND BELOW-GROUND. (FIELD EXPERIMENTS, 1944-45 AND 1945-46)

Position of inoculum	Distance from plants, in.	Av. number of plants killed	
		1944-45	1945-46
Aboveground, in pot buried at soil level	½	5.0	5.1
	1	4.8	2.3
	2	None	0.5
Below-ground inside inverted pot	½	5.0	5.5
	1	2.3	3.2
	2	0.5	1.3

As shown in Table III, killing of the plants resulted from spread of the mycelium above and below ground. Approximately the same number of plants were killed in both cases. No mycelium developed on the surface of the intervening soil in the below-ground series. Similar results were obtained in other experiments where the barriers and inoculum were placed so that the mycelium could spread from plant to plant in the rows.

The ability of the mycelium to spread upwards in the soil was studied by placing inoculum at depths ranging from 1 to 18 in. below the surface. Excavations were made at intervals along rows of alfalfa plants and at each staked

position a pinch of inoculum was carefully placed at the required depth in contact with the root of one plant. The average number of plants killed in a representative experiment (1942-43) was as follows:

Inoculum at surface	4.0
Inoculum at 1 in. depth	4.5
Inoculum at 3 in. depth	4.2
Inoculum at 6 in. depth	3.0
Inoculum at 9 in. depth	2.5
Inoculum at 12 in. depth	1.5
Inoculum at 15 in. depth	2.0
Inoculum at 18 in. depth	1.0

In this and other experiments killing took place over a smaller area with the deeper placement of the inoculum. However, even at the 18 in. depth, the plant in contact with the inoculum was usually killed. Severe rotting was not found at the point of inoculation on any of the roots in the above experiments. In all cases merely superficial lesions extended up the root to the crown, which was completely rotted. There was an apparent tendency for the mycelium to spread upwards through the soil in proximity to the roots and to cause injury only at the crown.

Distance of Spread

The distance of spread of the winter crown rot pathogen in alfalfa stands was determined by placing a small quantity of inoculum in the fall at staked positions in the center of plots or at intervals in rows of plants. Spread of the pathogen, as indicated by dead plants, was measured the following spring. As shown in Table IV, the average distance of spread of the killing varied considerably from year to year. The greatest spread (7.5 in.) occurred in 1942-43, when the damage in alfalfa fields was also particularly severe (Table I). It was least (2.7 in.) in 1943-44, when there was relatively slight damage in alfalfa fields.

TABLE IV

DISTANCE OF SPREAD OF THE WINTER CROWN ROT PATHOGEN AMONG ALFALFA PLANTS AND ACROSS BARE SOIL, AS INDICATED BY THE DEGREE OF KILLING (1942-46)

Winter	Average distance of spread of killing among plants, in.	Percentage of plants killed when inoculum was placed at various distances, in. from the rows							
		¼	½	1	2	3	4	5	6
1942-43	7.5	100	100	98	92	72	72	52	36
1943-44	2.7	100	50	0	0	0	0	0	0
1944-45	3.0	100	84	80	80	8	0	0	0
1945-46	4.5	100	100	100	58	20	3	0	0
Average	4.4								

In studying the distance of spread of the pathogen across soil unoccupied by plants, inoculum was placed in shallow trenches parallel to rows of alfalfa at distances ranging from ¼ to 6 in. Inoculations were made in the fall and the percentage of plants killed in each row was determined the following spring.

As shown in Table IV, the pathogen did not spread quite so readily across bare soil as among the plants in plots or rows. However, in the 1942-43 experiment there was killing in the rows 6 in. distant from the inoculum. The spread did not exceed ½ in. in 1943-44 and, as in the previous experiments, it was directly related to the severity of the damage in alfalfa fields.

Discussion

The widespread occurrence of the winter crown rot pathogen on forage crops and other hosts in cultivated and virgin land in Alberta indicates that it has been long established, and may even be native in this region. Prior to the present study, the damage caused by this pathogen was probably attributed to true winter killing or to other crown- and root-rotting fungi. This is quite understandable since the damage occurs at about the same time as winter killing, and isolation of the pathogen is made difficult by secondary invasion of the tissues by other organisms. The pathogen undoubtedly increases under field conditions, and is becoming more destructive with the increasing cultivation of alfalfa and other highly susceptible crops. The limitations of mycelial spread, however, appear to prevent the pathogen from spreading to any extent from field to field. Little is yet known about the manner in which it persists and spreads in nature or in the absence of a susceptible host.

It is evident that there are several stages in the development of winter crown rot. In the first place, the pathogen apparently requires a period of mycelial growth under suitable conditions before freeze-up in the late fall. If infection does occur during the fall period it is probably not important, since the plants are not susceptible until they reach a certain stage of dormancy. By this time the soil is usually frozen and there is no opportunity for infection until thawing starts in the late winter or early spring. The pathogen then resumes growth and may spread very rapidly and cause severe damage if conditions are favorable. It develops best in the natural cavities formed at the soil surface beneath a slowly melting snow cover. This suggested cycle of development ends when the snow melts, since the plants become decreasingly susceptible as growth begins and they lose their hardened and dormant properties. As the soil temperature rises, the pathogen also ceases to develop, and becomes dormant until conditions are again favorable for growth during late fall.

Environmental conditions at the time of the first spring thaw have a marked effect on the nature of winter crown rot damage and on its severity from year to year and in different localities. Thawing first starts at the surface of the soil, enabling the pathogen to grow and attack the crowns of the plants. If the snow melts slowly, the crowns may be completely rotted and the plants killed while the underlying soil is still frozen. Present evidence is that early

and rapid melting of the snow invariably results in lessened damage. This may explain the relatively slight injury that occurs in southern Alberta, where the warm Chinook winds may cause rapid melting of the snow at any time during the winter. A similar relationship between a slowly melting snow covering and increased snow mold damage in grasses and cereals was observed by Remsberg and Hungerford (6), and Wernham and Chilton (10) in studies on *Typhula* spp., and by Schaffnit (8) with *Fusarium nivale*. Soil temperature and moisture and other factors also have an important influence on disease development in relation to host physiology and require further detailed study.

Acknowledgments

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EFFECT OF CROP DEBRIS, PLANT ROOTS, AND CROP SEQUENCE ON THE MICROBIAL FLORA OF THE SOIL IN RELATION TO ROOT ROT IN CEREAL CROPS[1]

By L. E. Tyner[2]

Abstract

The decomposition of wheat, oat, or barley straw in soil caused a marked increase in the microflora, as indicated by plate counts. The oat straw compost yielded significantly more colonies of fungi than either the wheat or barley straw composts. The population of bacteria and actinomycetes was increased to about the same degree by all three of the composts.

In another laboratory experiment, wheat, oats, barley, and beans were planted in various rotations in pots of soil from field plots known to be heavily infested with the root-rotting pathogens *Helminthosporium sativum* P. K. and B., *Ophiobolus graminis* Sacc., and *Fusarium* spp. Fungi, mostly *Penicillium* spp. and *Mucor* spp., were up to 15 times more abundant from the rhizosphere of wheat roots than from the rhizosphere of oat, barley, or bean roots, regardless of crop sequence. It is assumed that the higher counts of saprophytic fungi obtained from the rhizosphere of wheat seedlings were directly correlated with the greater amount of dead root tissue on this crop, since disease on the wheat seedlings was more severe than on the other hosts. In a duplicate experiment in fallow soil, the rhizospheres of wheat, oats, barley, and beans yielded about equal numbers of fungi.

Introduction

In previous work (7), it was found that root rot on wheat seedlings was less in composts of soil with oat straw than with wheat or barley straw. It was thought that the microflora concerned in the decomposition of the oat straw depressed the root-rotting pathogens. In the present paper attempts were made to assess quantitatively the microflora in different straw–soil composts, and also to investigate the effects on it of both living and decomposing root systems of wheat, oats, barley, and beans.

Methods and Results

Effect of Cereal Straw Upon the Growth of Microorganisms

Finely chopped straw of wheat, of oats, and of barley was mixed with black soil to make 3% by weight of each mixture. The mixtures were then put into 1-gal. crocks; the moisture was adjusted to 20% content; and the crocks were covered with waxed paper and placed on a greenhouse bench. Two days afterwards, each crock was emptied, the compost thoroughly mixed, and a 100 gm. sample removed. The moisture content was again adjusted to 20% and the soil returned to the crocks. Samples were similarly removed every three weeks for seven successive periods.

Each 100 gm. sample was thoroughly mixed and a 40 gm. aliquot was taken; this was further subdivided into two 20 gm. portions, one of which was used

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to make dilutions. This portion was added to one liter of sterile tap water and shaken for five minutes. Ten milliliters was withdrawn and added to one liter of water; the mixture was well agitated. Of this concentration, 50 ml. was added to 150 ml. of water to make a dilution of 1 : 20,000 for counts of fungi, and 5 ml. was added to one liter of water to make a dilution of 1 : 1,000,000 for counts of bacteria and actinomycetes. In each case one milliliter of the final respective dilutions was added to seven replicated 10 cm. Petri plates and mixed with a synthetic agar medium for bacterial counts. When this medium was used for counts on fungi, it was supplemented with 0.25% boric acid to suppress bacterial growth (8). The plates were incubated at a temperature of 70° to 74° F. and the counts of fungi and of bacteria were made on the fourth and on the 10th day, respectively.

In addition to the use of soil–straw composts, dilutions were made in the above manner from crocks containing straw of each kind unmixed with soil. Counts of fungi and bacteria were made from these crocks on three different occasions, each at three-week intervals. All data appear in Table I.

TABLE I

NUMBER OF COLONIES OF FUNGI, BACTERIA, AND ACTINOMYCETES OBTAINED AT THREE-WEEK INTERVALS FROM DECOMPOSING STRAW AND 3% STRAW–SOIL COMPOSTS

Substrate	Fungi							Bacteria and actinomycetes						
	Thousands per gram							Millions per gram						
	Three-week intervals							Three-week intervals						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Wheat straw – soil	192	35	20	22	20	29	20	80	55	27	47	18	40	20
Oat straw – soil	272	320	178	90	84	146	69	71	84	43	45	16	43	15
Barley straw – soil	134	70	68	25	24	40	17	36	75	47	57	59	25	13
Soil	22	10	10	12	12	22	20	6	12	8	21	5	6	6
Wheat straw	272	312	137	—	—	—	—	70	53	19	—	—	—	—
Oat straw	1642	320	80	—	—	—	—	78	38	14	—	—	—	—
Barley straw	204	192	40	—	—	—	—	74	58	15	—	—	—	—

It will be noted that fungal colonies were most numerous in plates from dilutions of oat straw and oat straw composts. On the other hand, the number of colonies of bacteria seemed to be uninfluenced by the kind of straw. Colonies were always more numerous when straw was present in the soil than when it was absent, as in the controls. This indicates that the process of decomposition of plant debris is associated with a marked increase of both fungi and bacteria. The growth of fungi was greatly stimulated during the first three week period, and the growth of bacteria during the second period. Vandecaveye and Katznelson (9) also found that in the process of decomposition of organic matter the fungus group was more active than the bacterial

group in the initial stages of decomposition. The high numbers of fungi in oat straw or its compost suggest that this straw provided a more suitable medium for fungi than did wheat or barley straw.

Influence of Previous Cropping Practice on the Microflora of the Rhizosphere of Various Field Crops

Sufficient soil to fill 80 6-in. pots was taken in the fall from field plots that had been replanted continuously to wheat for 11 years, and that were known to carry a heavy natural infestation of root-rotting fungi. The 80 pots were separated into four groups of 18, with eight pots for controls.

In the first planting, 18 pots were planted to wheat, 18 to oats, 18 to barley, and 18 to beans, and the eight remaining control pots were left fallow. The pots were placed in large shallow trays on the greenhouse benches and watered from below to ensure a reasonable uniformity of moisture content. One month later, root-rot infection data were secured from the seedlings. The root systems were collected from all pots and freed of most of the adhering soil. Duplicate 10 gm. aliquots of the roots were weighed, one for making dilutions, and the other for computing on a dry basis the weight of roots and adhering soil. Ten grams of soil from the control pots were also taken for dilution. The dilutions were made from the 10 gm. aliquot of the roots that had been cut into segments of about 1 cm., and the plates were poured on the same day that the plants were lifted. The media and the incubation period were the same as in the last experiment. The numbers of fungi, actinomycetes, and bacteria were computed on a basis of the dry weight of the sample.

All pots were replanted on the day following the first harvest. The 18 pots that were planted to wheat in the first planting were replanted to wheat. Six of those planted to oats, barley, or beans, were planted to wheat, and the remaining 12 pots of each group were replanted to oats, barley, and beans, respectively. In the third planting all the 18 pots having wheat in the first and second plantings were replanted to wheat. Twelve of those having oats, barley, or beans were planted to wheat, and the remaining six pots in these groups were replanted to oats, barley, or beans, respectively. This planting sequence made it possible to secure data on the microflora of the rhizosphere from the following rotations: wheat, wheat, wheat; oats, wheat, wheat; oats, oats, wheat; oats, oats, oats; barley, wheat, wheat; barley, barley, wheat; barley, barley, barley; beans, wheat, wheat; beans, beans, wheat; beans, beans, beans; and the soil control. After the three plantings just described, the experiment was repeated, black Edmonton loam in fallow from the field being used instead of root-rot soil. All data obtained from this experiment, as well as those from the plantings in the root-rot soil, are shown graphically in Fig. 1.

Fig. 1 shows that very high numbers of fungus colonies were obtained from the rhizosphere of the roots of wheat plants grown in root-rot soil. Colonies were also very numerous from roots of wheat seedlings following an oat crop.

The increase was not so marked after barley or beans, and after two crops of beans there was apparently no increase on wheat roots. The average counts of fungi for the three plantings were 1550, 423, 146, 160, and 9 thousand per gram for wheat, oat, barley, and bean roots, and soil, respectively. The colonies from this soil comprised 64% *Mucor* spp., 29% *Penicillium* spp., and 7% miscellaneous spp. The disease rating varied directly with the observed trends in the fungus counts and was moderately high in all cases. This result was to be expected, as the soil in the field had been cropped to wheat for 11 years, which thereby favored an increase in wheat pathogens. On the other hand, the data indicated that organisms pathogenic to oats, barley, or beans were practically absent at the time of the first planting.

Average bacterial counts for the three plantings in root-rot soil were 183, 94, 264, 39, and 5 million per gram for wheat, oat, barley, bean roots, and soil, respectively. Bean and oat roots were much less favorable substrata for bacteria than barley or wheat roots. Except in the case of beans, replanting of the same crop favored increase in the bacterial population.

In the fallow soil the fungus colony counts were not excessively high, and there was a tendency for the count to decrease with successive plantings. The average numbers of colonies for three plantings were 235, 133, 178, 85, and 8 thousand per gram for wheat, oat, barley, and bean roots, and soil, respectively. The colonies comprised 48% *Mucor* spp., 46% *Penicillium* spp., and 6% miscellaneous spp. The contrast in counts between this soil and the root-rot soil was very marked in the case of wheat roots, but to a less extent in the case of oat roots.

TABLE II

INFECTION RATINGS OF WHEAT, OAT, AND BARLEY SEEDLINGS AFTER THREE SUCCESSIVE MONTHLY REPLANTINGS IN POTS OF SOIL FROM VARIOUS SOURCES

Soil sample	Infection, %								
	Wheat seedlings			Oat seedlings			Barley seedlings		
	Planting			Planting			Planting		
	1	2	3	1	2	3	1	2	3
After one oat crop	8	18	20	20	14	21	11	26	17
After one barley crop	25	12	23	17	22	28	15	11	16
After 12 wheat crops	25	23	33	4	9	19	4	21	23
Virgin soil	7	13	17	6	24	24	8	16	25
Under willow	2	4	8	6	17	15	6	22	20

The bacterial population was quite low in the fallow soil at the first planting, but the average counts for the three plantings were higher than those for the root-rot soil. The average number of colonies were 312, 204, 175, 134, and 2 million per gram for wheat, oat, barley, and bean roots, and soil, respectively. With the exception of two cases in which counts were unusually high on wheat roots, there was little difference in the population pattern for the different crops.

Infection ratings for seedlings grown in this fallow soil were low and averaged about the same for wheat, oats, and barley. It would appear that organisms pathogenic to the seedlings of these crops were not numerous in this soil.

It will be noted that fungi and bacteria were always more abundant in the rhizosphere of the seedlings than in the soil controls. Apparently the living roots stimulate the growth of microorganisms, and it is significant that the degree of stimulation may vary with the crops. Starkey (5), Clark (1), Timonin (6), Lochhead (2), and West and Lochhead (11) have reported on similar stimulations to microbial growth, in particular bacteria, from the presence of plant roots in the soil.

It seems that a complexity of factors influences the population of microorganisms in the rhizosphere. In the root-rot soil, conditions were very favorable for the growth of fungi on dead wheat roots, and probably the pathogens pave the way for other fungi, mainly *Penicillium* spp. and *Mucor* spp., by providing rotted tissue as food. In this connection data from a supplementary experiment showed that slightly over twice as many fungus and bacterial colonies developed from plated washings from the crowns and roots of moderately attacked wheat seedlings than from those containing only a trace of disease. None of these were root-rot pathogens. The bacterial colonies observed were 405 and 190 million per gram of dry root tissue, and the fungus colonies numbered 1.62 and 0.75 million per gram.

The number of fungus colonies obtained from the fallow soil was, by comparison, low, and did not increase with the replanting of the different crops. However, the bacterial population was relatively high in both fallow and root-rot soil, and it increased with replanting of the crops in both soils (Fig. 1). It may be that this effect of replanting on the bacteria is attributable to some growth stimulating substance left in the soil by the roots (6).

Relative Degree of Infestation of Various Soils by Root-rotting Fungi

It was decided to observe root-rot infestation as it might occur naturally in soils of the same type, but having different crop histories. Five such samples were secured for this purpose during October. One of these was obtained from a field plot that had just produced a crop of oats, another from a plot that had grown a crop of barley, and a third from a plot that had grown 12 continuous crops of wheat. The fourth sample was taken from unbroken virgin sod, and the fifth from under willow shrubs where no sod had formed. Four pots of each of these soil samples were planted with wheat, four with oats, and four with barley. The pots were placed in large shallow trays on greenhouse benches and watered from below. Data on infection were secured from three successive monthly plantings. The results appear in Table II.

The disease rating on wheat seedlings grown in soil after 12 crops of wheat was 25%, whereas the oat and barley seedlings were practically clean in the same soil. In the soil from oat stubble, the disease rating on the seedlings

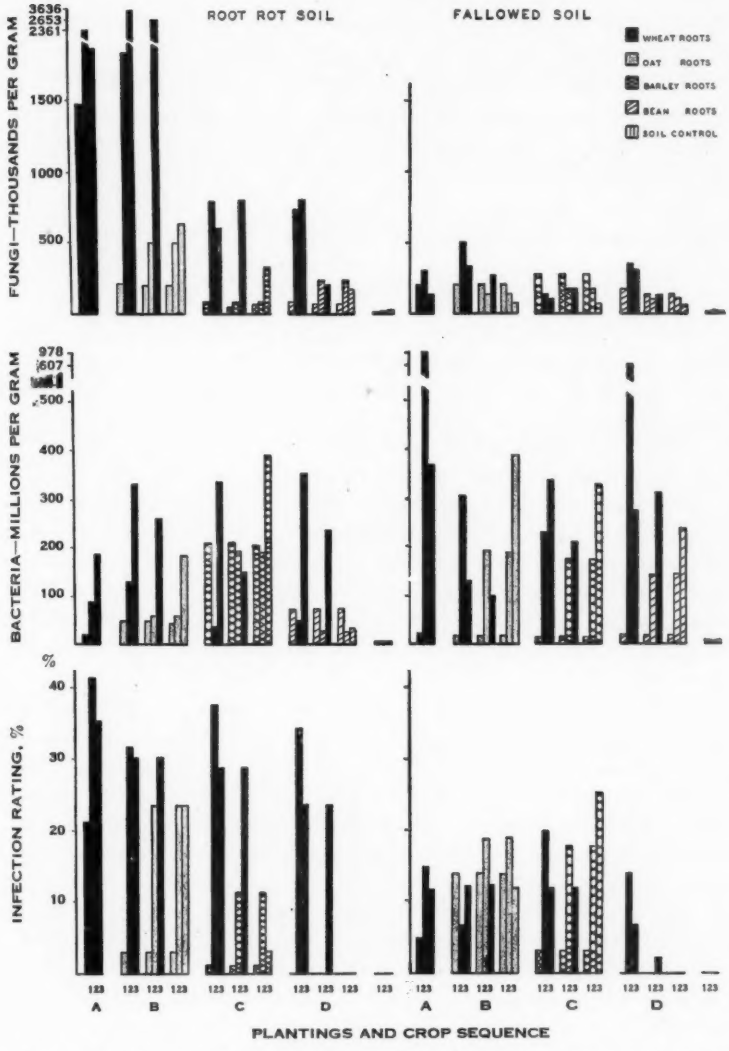

FIG. 1. *Numbers of microorganisms in the rhizosphere of wheat, oat, barley, and bean seedlings from three successive monthly plantings in various rotations; and the infection rating on the roots of the wheat, oat, and barley seedlings.*

1, 2, 3 = plantings.

A, B, C, D = wheat; oats–wheat; barley–wheat; beans–wheat rotations, respectively.

was 20%, as compared to 8% and 11%, respectively, on wheat and barley seedlings grown in this soil. On the other hand, in the soil sample taken from barley stubble the disease rating was 15% on the barley seedlings, whereas it was 25% and 17% on wheat and oat seedlings, respectively.

No explanation is offered of the tendency for the disease rating gradually to increase on the wheat, barley, and oat seedlings in the second and third plantings in the willow and virgin soils. Possibly pathogenic *Fusarium* spp. and *Helminthosporium* spp. were indigenous, or the disinfected seed still carried inoculum, or air- and water-borne spores found access to the soil during the experiment.

Discussion

A previous study (7) indicated that root rot on wheat seedlings grown in composts of soil with oat straw was consistently less than in composts with wheat or barley straw. In the present study it was found (Table I) that colonies of fungi, mainly *Penicillium* spp. and *Mucor* spp., were significantly more numerous in dilutions from decomposing oat straw or oat straw composts than in those from the wheat or barley straw or their composts. In view of these results it seems probable that in the previous study (7) the root-rot pathogens in the oat straw – soil compost were suppressed by the abundant development of such species in that compost. Sanford and Cormack (4) showed that *Penicillium* spp. were active in suppressing the pathogenicity of *Helminthosporium sativum*, and Sanford (3), in a review of the potential roles of association effects in plant disease, has indicated how pathogenicity may be accentuated or attenuated by the soil flora associations.

The present study has shown that fungus growth was greatly stimulated by the presence of roots, and, in particular, by wheat roots in a soil that had been cropped for 11 years to wheat in the field (Fig. 1). Infection ratings on the wheat seedlings grown in this soil were high, a result expected from the cropping practice mentioned. It might be considered anomalous that an abundant fungus growth in the oat straw compost (7) would reduce disease, whereas in the root-rot soil it would not. As already mentioned, bacteria, actinomycetes, and spores of certain fungi (*Penicillium* spp., and *Mucor* spp.) were greatly stimulated by the presence of roots. It is possible that the increase in the saprophytic fungi from the rhizosphere of wheat seedlings in the root-rot soil was a direct result of disease. Starkey (5) found by direct examination that microbial development was most extensive where dead root material was decomposing. Wheat roots even moderately infected with root rot show extensive lesions, not only in the region of the crown, but also at various places on the primary and secondary roots. Evidently this rotted tissue is a very favorable medium for the growth of fungi as well as bacteria. In general, there was a marked tendency for both microorganisms and root rot to increase with each successive planting of the same crop.

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USE OF THE INFRARED TOTAL ABSORPTION METHOD FOR ESTIMATING THE TIME COURSE OF PHOTOSYNTHESIS AND TRANSPIRATION[1]

By G. W. Scarth,[2] A. Loewy,[3] and M. Shaw[4]

Abstract

The type of apparatus designed by Dingle and Pryce for determining the concentration of carbon dioxide in air by non-spectroscopic measurement of its absorption of infrared has been modified so as to increase its accuracy greatly. Less than one part by volume of carbon dioxide in a million of air can now be measured at speeds ranging from 10-min.-interval to continuous readings. Water vapor can also be measured accurately and continuously.

Examples are given of the use of the technique in following the time course of photosynthesis and transpiration, including a correlation of the courses of these processes with leaf temperature and stomatal movement during rapid wilting of a detached leaf of *Pelargonium*.

Introduction

Since carbon dioxide gas and water vapor absorb infrared and are the only constituents of normal air that do so, and since this affords a rapid and speedy means of estimating their concentration either in streaming or stationary air, it is rather surprising that so little use has been made of the technique in plant physiology. To the writers' knowledge the only application is that of McAlister (2) who developed it with great success to follow the time course of photosynthesis, including such transitory phenomena as 'induction', which appears for a few minutes at the start of a light period and 'dark pick up' of carbon dioxide, which occurs even more briefly after the light is cut off. McAlister's technique showed remarkable sensitivity as well as speed, measuring as low as one part of carbon dioxide to a million parts of air at intervals of a few seconds. The main drawback is the considerable complexity and cost of the apparatus required. To separate the absorption effect of carbon dioxide from that of water vapor he used an infrared spectroscope having rock salt optical parts. This allowed a narrow band of 4.2 to 4.3μ wave lengths, which lies at the peak of the absorption band of carbon dioxide and outside that of water vapor, to be directed upon a vacuum thermocouple. The small current generated in the thermocouple required a sensitive galvanometer to measure it, and for certain purposes a photronic relay and secondary galvanometer in addition (3).

A simpler technique for measuring absorption by carbon dioxide was introduced by Dingle and Pryce (1). It dispenses with the spectroscope and requires less sensitivity in the detecting apparatus. With this method the

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carbon dioxide effect is isolated by drying the air before it enters the absorption chamber. Though Dingle and Pryce do not mention it, the method can also be applied to water vapor without even the necessity of removing carbon dioxide. Here the total absorption by the gas is measured, and a broad beam of radiation can be directed upon a thermopile. Hence less galvanometer sensitivity is needed. Another advantage is the relative independence of fluctuation in air temperature, which, while it alters the position of the absorption band, has little effect on the amount of absorption.

On the other hand, the absence of spectroscopic (or other) screening allows the thermopile to be heated by 'useless' radiation, which may be of much greater intensity than the part that is to be measured. Dingle and Pryce counteracted this effect by balancing their sampling absorption chamber and its thermopile against a similar control chamber and second thermopile. The differential current produced by the opposed e.m.f.'s is then determined by the difference in concentration of carbon dioxide (or water vapor) in the respective chambers. As a common source of infrared they used the flame of a Meker burner.

The only use of the apparatus that these authors report is in measuring the absorption by different concentrations of carbon dioxide made up for the purpose of constructing a calibration curve, and it is not clear whether the low galvanometer sensitivity they employed was determined by error in making up the supposed concentrations or in measuring them. If the latter, the apparatus had only 1/13 the sensitivity to carbon dioxide of McAlister's. Moreover, in measuring photosynthesis at the usual rate of air flow, which allows no more than one-fifth of the total carbon dioxide to be removed by the plant, the possible error when photosynthesis is at its maximum would be about 33%.

Since, however, there appeared to be no inherent reason why the total absorption method should be so greatly inferior in sensitivity to the spectroscopic one, we decided to investigate its possibilities. Starting with equipment essentially the same as Dingle and Pryce's, we found that its capacity to measure carbon dioxide was, in fact, no greater than the above estimate. However, by making various changes that, though not drastic individually, add up to produce an impressive total effect, we have increased stability to such an extent that a galvanometer sensitivity more than 20 times that used by Dingle and Pryce is profitable in making measurements. The apparatus is now capable of measuring carbon dioxide with even greater accuracy than McAlister's, though with less speed, and of measuring water vapor more accurately than is ever likely to be required. It has some obvious defects, most of which could be remedied easily in setting up new equipment, and it appears capable of much further development along different lines for particular purposes.

The present paper describes the apparatus as recently used by us and gives examples of the application of the technique to physiological studies. These comprise, as far as we know, the first reported physiological use of the total

absorption method, and the first application of any infrared absorption method to measurement of transpiration.

The Apparatus

A description of the principal parts of the apparatus follows and a diagram of the whole assembly is shown in Fig. 1. It differs from the original form of the apparatus mainly in the source of infrared and in the sensitivity of the galvanometer.

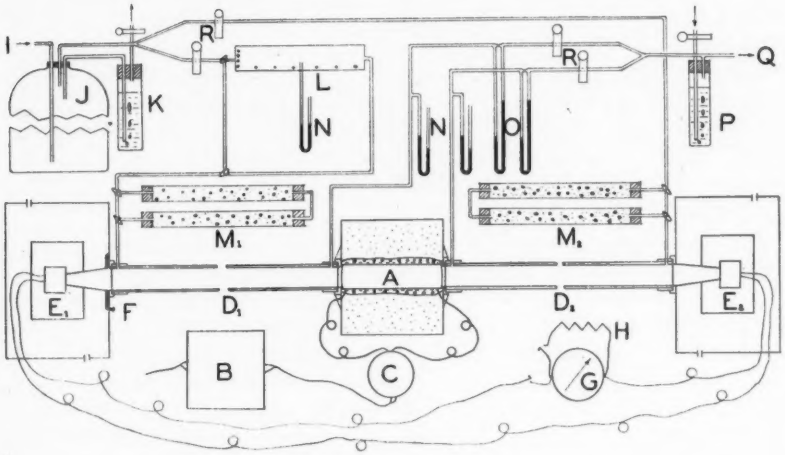

FIG. 1. *Diagram of the apparatus.*

A — Heater, or infrared radiator, in median section.
B — Constant voltage transformer.
C — Variac.
D_1 and D_2— Infrared absorption tubes.
E_1 and E_2— Thermopiles.
F — Iris diaphragm.
G — Galvanometer.
H — Damping resistance.
I — Position of electric air pump.
J — Air reservoir for regulating flow.
K — Overflow valve.
L — Leaf chamber.
M_1 and M_2— Drying tubes.
N — Manometers.
O — Flowmeters.
P — Inlet valve.
Q — Position of constant head suction pump.
R — Adjustable clamps.

The *heater* (*A*), source of infrared, is an electrically heated quartz tube filled with carbon dioxide. The tube (18 × 2.6 cm.) is wrapped with 12 ft. of flat nichrome wire, having a resistance of 1.6 ohms per foot, and by asbestos string to hold the wire in place. This heater is enclosed by a couple of fire bricks and is set in alignment with the two absorption tubes. A collar of

asbestos cement covers the junction with the latter. With the aid of a constant voltage transformer (input load 100 to 120 v., 7.5 to 12 amp.: output 115 v., 1.75 amp.) and a variac, the heater is supplied with a very steady current. This current of 65 v. and 3 amp. produces a temperature of about 800° C. at the middle of the quartz tube.

The *infrared absorption tubes* (D_1 and D_2) are of stainless steel, polished inside, 190 cm. in length and 2.56 cm. internal diameter. Their ends are fitted with brass sleeves and screw-on caps that hold mica windows in place. As there is considerable absorption of useful wave lengths by mica, the sheets are made only thick enough to withstand buckling under the highest pressure difference that is likely to be set up, about 20 cm. of oil. Near each end of the absorption tubes small lateral tubes for inlet or outlet of air are inserted.

The *thermopiles* (E_1 and E_2) are the Moll small surface type, which has 16 thermocouples. Their conical reflectors just fit the ends of the absorption tubes. The sensitivity of the two thermopiles was found to be unequal, in the ratio of 6 to 5. They are therefore exposed to radiation intensities of approximately inverse ratio. An iris diaphragm (F) interposed in front of one of them facilitates the balancing. Each thermopile is enclosed in a copper box and this in a larger cardboard one through which air can circulate. The use of reflectors to concentrate the rays has this advantage over the use of larger thermopiles that it does not amplify the effect of changes in external air temperature.

The *galvanometer* (G) is a Cambridge moving magnet one, shielded pattern, resistance 22 ohms. Its maximum sensitivity, about 4000 mm. per μa. at 1 meter, and even the catalogued sensitivity of 1200 mm. is far beyond requirement, but the sensitivity can be varied without damping. For photosynthesis measurement sensitivities ranging from 500 to 650 mm. per μa. are found to be convenient, for transpiration less than 1/10th of that. In changing briefly from one type of measurement to the other sensitivity was reduced by damping rather than setting the galvanometer.

Support for all parts of the apparatus is firm and steady. The galvanometer is set on a concrete pillar resting on the concrete floor of a basement room. The heater, absorption tubes, and thermopiles, after being aligned for maximum sensitivity, were securely fixed to a rigid wooden base. This not only prevents accidental displacement of parts but allows the whole to be moved as one piece.

The *air stream system* is represented in the diagram in a somewhat condensed form. Fresh air is brought in from outside the building by means of an electric pump at I. After passing a pressure regulator (and also certain air conditioning devices not included in the diagram) the stream divides. Each branch in its course travels through, or may by-pass, a set of drying tubes (M) filled with magnesium and barium perchlorates, then through one of the absorption tubes (D), past a manometer, and through a flowmeter. In addition one branch can be routed through the leaf chamber (L) situated

anterior to the drying tubes. The stream, united again beyond the flowmeters, passes through another regulator and is drawn off by a constant head suction pump at *Q*.

The rate of flow and pressure gradient in the common stream are roughly adjusted by varying the openings in the overflow valve (*K*) and inlet valve (*P*) and are more precisely adjusted in the branches by means of two pairs of hinge clamps (*R*) that embrace sections of pressure tubing. This multiplication of controls is due to the necessity of maintaining the leaf chamber at atmospheric pressure and also equalizing the pressure in the two absorption tubes at any desired rate of flow.

The *leaf chamber* (*L*) consists of a Petri dish lid resting on a glass plate, the edge being sealed with plasticine when the leaf is in place. The leaf used in the experiments described later was that of *Pelargonium zonale*. A notch in the edge of the lid accommodates the petiole and admits the wires of a thermocouple. Fitted into the lid are inlet, outlet, and manometer tubes. A wire grid supports the leaf and a gauze baffle at the inlet distributes the air evenly. It is not necessary to describe here the means whereby temperature and humidity are controlled, but it may be mentioned that the measurement of environmental conditions is a subsidiary use of the apparatus. It provides a rapid and easy means of measuring the vapor pressure in the air stream, its galvanometer connected with thermocouples gives the temperature of the leaf or air and in conjunction with a photoelectric cell reads the light intensity at the level of the leaf. Thus the operator can observe at will on his galvanometer scale the course of photosynthesis, transpiration, or respiration and the environmental conditions that normally control the rate of these processes.

Calibration

In calibrating the apparatus for carbon dioxide measurement 'normal' dried air was run through the control absorption tube, and normal air mixed with carbon dioxide free air (both dried) in known proportions through the

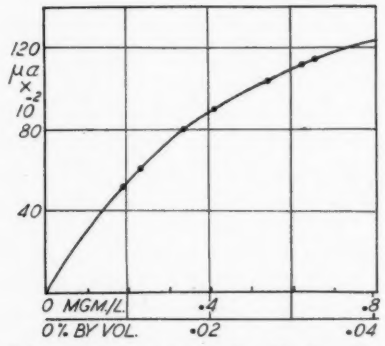

FIG. 2. *Galvanometer current vs. carbon dioxide content of the air.*

'experimental' tube. The carbon dioxide content of the normal air was measured by absorption in baryta solution and titration. The mixing was done with the aid of carefully calibrated flowmeters. In plotting the calibration curve (Fig. 2), the deflection obtained with carbon dioxide free air was

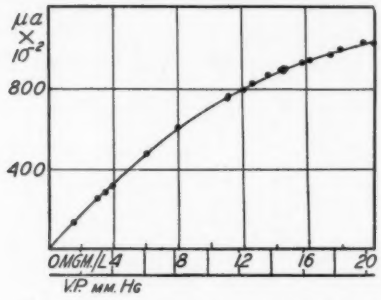

FIG. 3. *Galvanometer current vs. moisture content of the air.*

made the zero point and deflection is converted to current flowing through the galvanometer. This allows the curve to be used for any galvanometer sensitivity and any concentration of atmospheric carbon dioxide with a minimum of calculation.

In calibrating for water vapor most of the vapor pressures used were those of normal air on different days, the values being calculated from sling psychrometer measurements. Vapor pressures below 8 mm. Hg were obtained by mixing normal and dried air. The measurements of galvanometer deflection were made with dried air as a control. The calibration curve (Fig. 3) shows the current produced by different moisture contents expressed both as vapor pressure in mm. of Hg and as milligrams of water per liter of air at N.T.P.

Operation and Performance

After the heater is turned on and air stream set in motion about 45 min. has to elapse before equilibrium is reached. Then, with the same air flowing through both absorption tubes, the galvanometer deflection, or 'apparatus zero' as we shall call it, is noted. This commonly fluctuates narrowly and slowly about the galvanometer zero, but occasionally it shows a protracted, steady one way drift—the result of a recognizable asymmetrical action of radiation or air temperature on the thermopiles. The fluctuating type of zero shift is negligible at the low sensitivities used for measuring transpiration, but for accurate measurement of photosynthesis it usually renders necessary the determination of apparatus zero alternately with experimental readings. During periods of fairly constant room temperature, however, satisfactory continuous records of photosynthesis are obtained even at high sensitivities.

The balancing for purpose of zero determination has to be done with the same kind of air in the tubes as that which occupies the control tube during

measurement, that is, dried air for photosynthesis and normal air for transpiration. During the course of measurement of photosynthesis the zero is checked by switching the air past the leaf chamber for long enough time to flush out the measuring tube completely. It is often more convenient to use the respiration level as a base line, darkening the leaf for a few minutes.

The usual type of fluctuation now encountered is illustrated in Fig. 4 where the irregularity in the photosynthesis record is known from other measure-

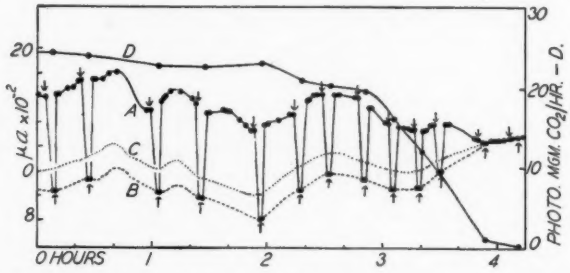

FIG. 4. *Photosynthesis rate of a wilting leaf vs. time: type of record obtained with an average degree of zero fluctuation.*

Curve A — Galvanometer current with leaf exposed to the light.

B — Galvanometer current with leaf darkened, leaf temperature unchanged: respiration level.

C — Probable apparatus zero level estimated from B and respiration rate.

D — Rate of gross photosynthesis of the leaf (right hand scale), calculated from Curves A, B, and from Fig. 2.

ments to correspond to that of the apparatus zero. Fig. 6 illustrates a relatively steady record with only one swerve (in the dark period) that can be ascribed to zero shift.

As an indication of the sensitivity of the apparatus to carbon dioxide, the current generated by 'normal' air (.03% carbon dioxide) vs. carbon dioxide free air (both dried) is 1.10 μa. With the galvanometer set so that this current produces 650 mm. deflection at 1 meter, individual readings can be made accurately to within 1 mm. and readings taken before and after zero determination, if not identical, do not differ more than 1 mm. as a rule. The average of the two measurements, therefore, is likely to be accurate to within 1 to 2 mm. representing a concentration of 0.5 to 1 $\times$ 10^{-4} % carbon dioxide. In general, a variation of less than one part of carbon dioxide per million (by volume) can be detected in normal air.

In measuring photosynthesis or transpiration by any gas stream method the rate of air flow is a factor. The error in measuring the latter is probably greater than in measuring concentration of gas by infrared absorption and will affect the accuracy of calculated *absolute* rates of either process. But, as regards *relative* rates, the rate of flow is so steady that little error is introduced by assuming it to be constant.

The *speed* of measurement as applied to photosynthesis is commonly limited by the time consumed in determining the apparatus zero between readings—about six minutes with the air stream flowing at 40 liters per hour.

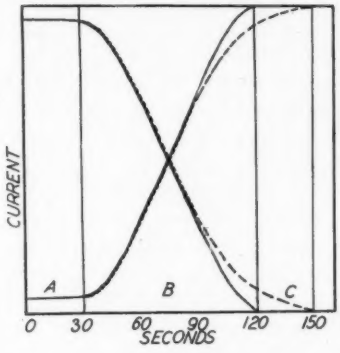

FIG. 5. *Apparatus lag in recording a sudden change in moisture content of the air made at zero on time scale. Flow 40 liters per hour.*

Continuous curve—Change made at outlet of leaf chamber.
Broken curve—Change made at inlet of leaf chamber.
Period A — Initial or transit lag.
B — Absorption tube lag.
C — Leaf chamber lag.

When the only zero determination, if any, necessary is that of the galvanometer, true speed of measurement is still limited by the time required for complete flushing out of the 'experimental' tube, because changes in the composition of the air occurring in less than this length of time are, as McAlister puts it, integrated or 'smeared out' in measurement.

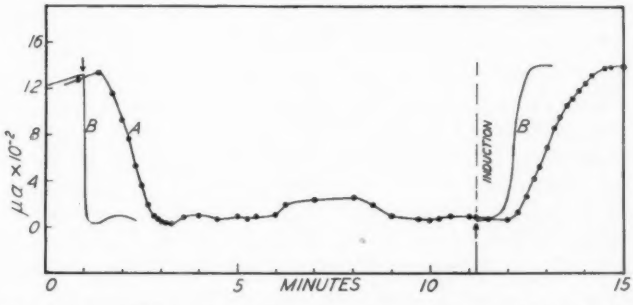

FIG. 6. *Course of photosynthesis following a sudden change from light to dark and from dark to light; ↑ light out, ↓ light on.*

Curve A — Galvanometer current vs. time as recorded.
B — Portions of Curve A corrected for apparatus lag.

It is possible, however, to correct for lag and 'spread' produced by the apparatus itself. Fig. 5 shows the course of galvanometer deflection that followed an abrupt change in the composition of the air produced (*a*) at the exit and (*b*) at the entrance of the leaf chamber. There is an initial lag of 30 sec. before any effect appears, the time required for the air to travel from the leaf chamber to the absorption tube. The only effect of this lag on measurement is to delay it. The slope that follows represents the progressive displacement of one type of air by the other in the absorption tube. The continuous curve flattens out about 90 sec. after it begins to fall, which is practically the time required to empty this tube as calculated on the basis of volume. The broken curve 'tail' extending over a further 30 sec. or so represents the emptying of the leaf chamber, delayed because of its shape by the formation of turbulent eddies. This sloping curve presents not only a lag but also a 'spread' of the event, lasting only a few seconds, which it records. Applied, however, to correct the curve of photosynthesis in Fig. 6 it allows a truer one of the time course to be drawn. The corrected curve shows clearly the 'induction' period that follows the start of illumination and indicates also that uptake of carbon dioxide does not cease abruptly on darkening.

While it is stretching the speed capacity of the apparatus in its present form to use it for the resolution of such rapid changes as are indicated in the previous experiment, it is admirably adapted for following those that are naturally timed in minutes rather than seconds. As an example, Fig. 7 shows what has probably never been measured before, the correlated time courses of transpiration, leaf temperature, photosynthesis, and stomatal movement during the rapid wilting of a leaf that, while exposed to a stream of dry air, was deprived of its water supply by cutting of the petiole. Two experiments, made on adjoining leaves of the same plant, are recorded here. In one, transpiration and leaf temperature were measured concurrently and in addition small strips were torn from the edge of the leaf at intervals for absolute alcohol fixation and stomatal measurement. Under constant external conditions the time curves of transpiration and leaf temperature always form mirror images of one another when plotted on an appropriate scale, so that leaf temperature can be used as an indirect measure of transpiration. Accordingly photosynthesis and leaf temperature were followed together in the second experiment.

Comparing the curves in Fig. 7A, we see that transpiration and stomatal opening increase slightly for a few minutes after the petiole is cut. (Passive opening of stomata at incipient wilting has frequently been recorded by other methods.) Then, for a time, transpiration drops more rapidly than stomatal closure, indicating some non-stomatal form of internal control. Later the two curves follow a parallel course of decline, and transpiration flattens quickly into the cuticular rate at the moment that stomatal closure is completed.

The photosynthesis curve in Fig. 7B shows a brief lag but no rise in its response to cutting. The decline that follows is rapid and parallels stomatal closure. At the completion of closure net photosynthesis is zero and gradually thereafter gross photosynthesis falls below the rate of respiration. To

correlate the two experiments, the inflections in the respective leaf temperature curves, which mark completion of closure, are synchronized in Fig. 7C. It appears that drop in rate of photosynthesis takes the lead of stomatal closure.

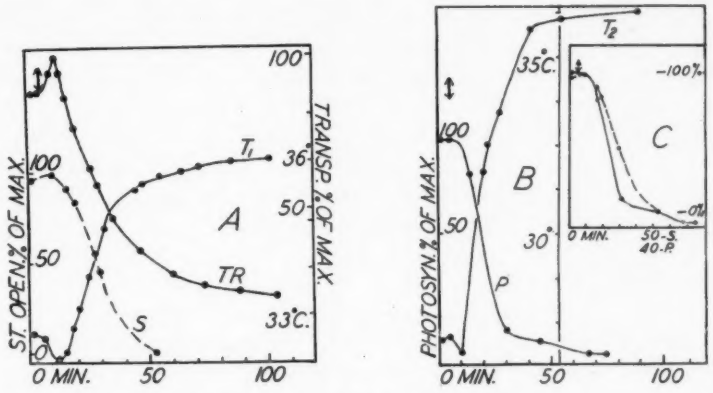

FIG. 7. *Changes during rapid wilting of a leaf;* ↕ *petiole cut.*

Tr — transpiration; S — stomatal aperture; P — photosynthesis; T_1 *and* T_2*— leaf temperature.*

Figs. A and B are separate experiments. Fig. C shows S from A and P from B plotted on different time scales so as to make the inflections of T_1 *and* T_2 *(closure of stomata completed) synchronize.*

The final 'exhibit' selected from our records of time courses is apparently a manifestation of physiological rhythm. While the records of transpiration and photosynthesis in Fig. 8 are not concurrent and do not apply to the same leaf, we have evidence that when one of the processes fluctuates in this way the other does so too. In each of the records shown here the light was turned on at its start and during the rest of the period external conditions remained practically constant. Owing to the relative infrequency of the photosynthesis readings the exact shape of the lower curve is uncertain but, as in other records of this type, the position of the points is at least compatible with as smooth and regular fluctuation as is clearly demonstrated for transpiration. This particular record of photosynthesis was chosen because it shows the oscillation fading out and reviving vigorously under uniform external conditions. In other cases a change of light intensity has induced renewal of oscillation after its complete cessation. On the basis of our present experience the occurrence of the phenomenon is quite unpredictable. Though not common neither can it be called rare. No doubt the oscillations in stomatal movement that have been reported are another aspect of the same phenomenon, but where the basic rhythm is seated has yet to be determined.

The apparatus is useful, and has been used, for quite another kind of purpose than the above set of examples. It serves to escape the effect of time rather than to observe it when applied to measuring transpiration or

photosynthesis under varied conditions before any appreciable stomatal change has had time to occur. But it seems unnecessary to multiply examples further to demonstrate the value of the technique.

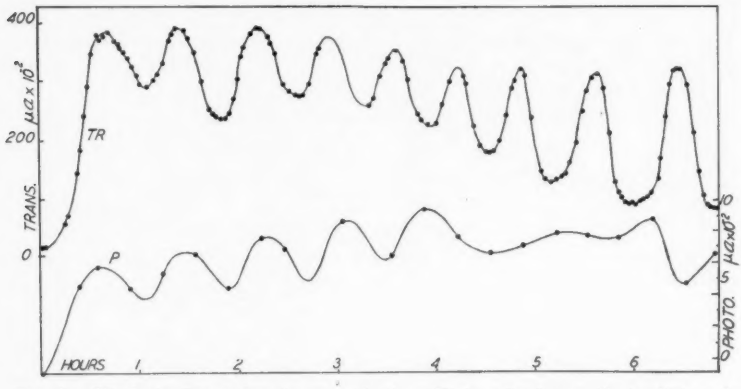

FIG. 8. *Rhythmic fluctuation in transpiration (Tr) and photosynthesis (P) under constant external conditions.*
The two curves are not directly related, being obtained with different leaves and at different times.

Defects and Their Remedy

Certain obvious defects that we have not yet had the opportunity to correct should be pointed out.

A fault of general design is the wide separation of the thermopiles, which renders them very liable to be subjected to differential changes in air temperature. This is found to be one of the most important causes of drift in apparatus zero. A better arrangement of parts would be to place the absorption tubes and thermopiles side by side, enclosing the latter in the same box. The present type of heater could easily be adjusted to suit the change by giving a U shape to the quartz tube.

Another defect, due to accident rather than design, is the asymmetry of the apparatus as shown by shifting of the zero when air temperature changes symmetrically with respect to the thermopiles or when a switch is made from normal to dry air in both tubes. The main cause of this asymmetry, at least in relation to external factors, is no doubt the marked inequality of sensitivity shown by our thermopiles. Another cause of the upset of balance that is produced by a change of air in the tubes may be a difference in the proportion of the various wave lengths emitted by the respective ends of the heater. As no precaution was taken to make the quartz of equal thickness at these points, asymmetry with respect to quality of radiation is not unlikely.

In the quality of this radiation, too, there is much room for improvement. As compared with the Meker burner, the heater has about one-third of the efficiency in producing wave lengths absorbed by carbon dioxide and three or

four times the efficiency in those absorbed by water vapor. But the reverse ratio would be more nearly ideal for the dual purpose of measuring both photosynthesis and transpiration. Whether due mainly to the weakness of the hot carbon dioxide source or to the relative opacity of quartz to wave lengths above 4 μ, much of the radiation, as we have found, comes from the surface of the quartz itself. (Hence the need for shielding the ends of the tube from air currents.) Improvement in the present type of heater might be effected by giving it thinner end windows of equal thickness.

Finally, on more theoretical grounds it is suggested that the efficiency of the apparatus would be increased by reducing the ratio of length to width of the absorption tubes—though the optimum volume will depend on the balance of speed and accuracy of measurement demanded for various types of investigation.

The relation of absorption to length of tube with carbon dioxide concentration constant must be similar to that of absorption (galvanometer current) to the amount of carbon dioxide (or water) in a tube of fixed length. The latter is shown, of course, in our calibration curve—which incidentally Dr. Rowles has found to be of exponential shape (cf. Fig. 9). If there were total reflection

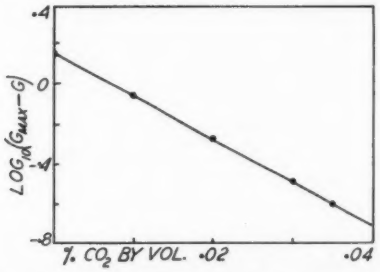

FIG. 9. *Logarithmic plot of carbon dioxide calibration curve, Fig. 2.* G_{MAX} *= 1.43 μa. galvanometer current, to which the calibration curve is asymptotic.* G *= galvanometer current as in Fig. 2.*

inside the tube it would seem that the respective curves should be practically identical and equivalent to that of the absorption of a parallel beam by different thicknesses of medium. Taking the carbon dioxide calibration curve, therefore, as representing this hypothetical case of identity, and substituting percentage of present tube length for concentration of carbon dioxide (.03% carbon dioxide = 100% length), the galvanometer current produced by removing *all* the carbon dioxide from one tube is shown on the graph. As used for measuring photosynthesis, however, a more useful criterion of sensitivity in relation to tube length is the effect of removing a small proportion, say 1/10th, of the atmospheric carbon dioxide. This too can be estimated from the calibration curve. Some relative values are shown in the following Table I.

Under actual conditions the deflection percentages in the shorter tubes should be somewhat higher even, as this calculation assumes that no radiation is lost to the walls of the tube in transit.

TABLE I

APPROXIMATE RELATION BETWEEN GALVANOMETER DEFLECTION AND TUBE LENGTH IN PERCENTAGE OF PRESENT VALUES

Length of tube	Deflection with all CO_2 removed	Deflection with 1/10 of CO_2 removed
100	100	100
50	67	90
25	40	70

The most efficient way, therefore, of increasing speed of measurement is to reduce the volume of the absorption tubes by shortening them. Any other method involves a loss in sensitivity proportional to the gain in speed.

Moreover, for the many purposes for which the present speed of measurement (with zero determination eliminated) is adequate, greater sensitivity to carbon dioxide can be obtained by widening the tubes in proportion as they are shortened. Assuming that the diameter of the heater is increased to fit, that its ends still emit the same intensity of radiation per unit area, and that the thermopile reflectors are enlarged to collect the full width of the 'beam', tubes of one-quarter the present length and four times the cross sectional area would increase sensitivity to small changes in carbon dioxide concentration 2.8 (4×0.7) times without any increase in sensitivity to external temperature changes.

No doubt further improvement could be made by more radical departures from the present design, but this is a subject for the physicist and outside the scope of this discussion.

Conclusion

The infrared total absorption technique as now improved, and still more as likely to be further improved by such changes as have been indicated, possesses unique value for studying the time course of photosynthesis and transpiration, the influence of external factors on the rate of these processes uncomplicated by change in stomatal aperture, and other relationships requiring speed of measurement for their estimation.

As the equipment is not very expensive, nor its manipulation difficult, the method seems worthy of widespread adoption.

Acknowledgments

Grateful acknowledgment is due to Prof. W. Rowles of Macdonald College for the loan of apparatus and for much valuable advice; also to the Department of Entomology, Macdonald College, for the use of laboratory space.

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